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Field Studies with Feed and Bolus Formulations of Dow ET-57 for Control of Cattle Grubs[1,2]

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ABSTRACT

Dow ET-57 in a bolus and as an additive in a feed formulation was tested in the fall of 1956 against cattle grubs, *Hypoderma* spp.

Four hundred seventy-nine Hereford steers and heifers were used, of which 235 head were located at the Fort Hays Branch Agricultural Experiment Station, Hays, Kansas, and 244 head were located at the Gooch Mills Juniata Experimental Ranch, Manhattan, Kansas. All treated animals were given 110 mg. of Dow ET-57/kg. of body weight. There were no consistent differences in gains in weight of the treated and untreated animals. Grub counts were made monthly or every 28 days in accordance with the cooperator's weighing schedule and showed a significant decrease in the number of grubs in the treated animals.

At the Fort Hays Branch station, 118 animals were treated with boluses and showed an average increase of 0.5 grub per animal. The 117 untreated animals showed an average increase of 11 grubs per animal. At the Juniata ranch, 80 head were bolused and showed an average increase of 0.38 grub per animal. Another 80 animals were treated by adding the insecticide to the feed and showed an average increase of 0.25 grub per animal. The 84 untreated animals showed an average increase of 3.2 grubs per animal.

Blood cholinesterase investigations were conducted on 30 animals, 10 of which were treated with boluses, 10 were treated by feed and 10 were used as controls, and showed that Dow ET-57 produced a marked depression of enzyme activity. Toxic symptoms were not observed in any of the experimental animals.

The availability of systemic insecticides for the control of cattle grubs, *Hypoderma* spp. has recently become a fact. The use of ET-57 in a bolus for oral administration to livestock, while quite effective, requires individual handling of the animals which may in some cases be difficult to accomplish. In the fall of 1956, the series of field experiments reported here was undertaken to investigate the feasibility of using ET-57 as an additive in a feed formulation. Although these treatments were initiated rather late in the season to anticipate any marked advantage in weight gains in the treated animals, detailed records of weight performance were maintained and are included, in part, in this paper.

PROCEDURE.—Four hundred seventy-nine Hereford steers and heifers were used. Two hundred thirty-five head were located at the Ft. Hays Branch Agricultural Experiment Station, Hays, Kansas, and 244 head were located at the Gooch Mills Juniata Experimental Ranch near Manhattan, Kansas.

Grub counts were made monthly or every 28 days in accordance with the cooperator's weighing schedule. The grubs were located in the backs of the animals by palpation and the location plotted on modified Riedel Charts (Riedel *et al.* 1955).

Ft. Hays Branch Station.—These cattle included 125 head of steer calves obtained from northeastern New Mexico during the fall of 1956, and 110 yearling steers which had spent the previous heel fly season at the station.

The steer calves averaged about 455 pounds on November 8, 1956 when 63 of them were selected at random and treated with ET-57 in boluses at the rate of 110 mg./kg. body weight. The remaining 62 untreated calves served as controls.

The yearling steers were divided into lots containing 50 and 60 head, each. Half of the 50-head lot was treated on November 9, 1956 and half of those in the 60-head lot were treated on January 10, 1957. Both lots were treated with ET-57 boluses at the rate of 110 mg./kg. body weight. These steers ranged from 1,000 to 1,200 pounds in weight.

Juniata Ranch.—These experiments were superimposed on nutritional experiments. The 244 head of cattle were placed into lots by the cooperator according to weights, but at random as far as grubs were concerned. All were from central Kansas and averaged about 500 pounds in weight at the time of treatment. Each animal received only one ET-57 treatment. All animals were weighed and surveyed for grubs on December 12, 1956 and every month thereafter through April, 1957.

On December 11, 1956, 80 head were treated with ET-57 in bolus form at the rate of 110 mg./kg. body weight. On December 12, 1956, an additional 80 head were fed a

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pelleted feed containing ET-57 (Dow formulation M-941 J-10-25-27), an emulsifiable concentrate. The feed was made available to the animals at the rate of 2 pounds per animal in regular feeding bunks. It was formulated to give an average treatment rate of 110 mg./kg. for a 500-pound calf consuming 2 pounds of feed. The remaining 84 head served as controls.

Preparation of the feed.—Two hundred fifty pounds of 32% range cubes containing ET-57 formulation M-941 at the rate of 12.26 grams of actual ET-57 per pound of finished feed were pelleted into ¼ inch pellets on December 4, 1956 at the Salina mill of the Western Star Milling Company. This feed was formulated by adding 13 pints of M-941 to small batches of feed and mixing by hand. The hand-mixed feed plus M-941 totaled 238.25 pounds. This was brought to a total of 250 pounds by adding more carrier feed.

After hand mixing, the 250 pounds were put into a shell mixer for 20 minutes. The formulated feed was then removed from the mixer and run through a screen to break up balls that had formed. The formulation was then returned to the shell mixer for another 5 minutes. The formulated feed was then pelleted, cooled, bagged, and shipped to the Juniata Experimental Ranch for feeding.

Samples for laboratory analysis were taken immediately from the pellet machine and stored in a deep freeze for subsequent insecticidal analysis.

Cholinesterase investigations.—Blood cholinesterase investigations were conducted on 30 animals from the Juniata herd. Ten of these animals were treated with boluses, 10 were treated by feed, and 10 were used as controls. The first samples were taken on December 11, 1956. The subsequent samples were taken 3, 5, 13, 22, and 40 days after the first sampling.

The blood was collected in 10-ml. screw-cap vials, heparinized and stored in a refrigerator until analyzed. A modified Hestrin method (Robbins 1956 personal communication) was used to determine cholinesterase inhibition. These 30 animals were surveyed for grubs each time blood samples were taken. A separate study was made of the grub data from these animals.

RESULTS AND DISCUSSION.—*Grub Studies.*—At the Hays Branch Station, the 118 animals that were treated (table 1) showed an increase of 59 grubs with an average increase of 0.5 grub per animal. The 117 used as controls showed an increase of 1,292 grubs with an average increase of 11 grubs per animal.

At the Juniata Ranch (table 1), already stated in "Procedure," the 80 animals which were bolused showed an increase of 30 grubs with an average increase of 0.38 grub per animal. The 80 animals that were given ET-57 in their feed showed an increase of 20 grubs with an average increase of 0.25 grub per animal. The 84 untreated animals used as controls showed an increase of 269 grubs with an average increase of 3.2 grubs per animal.

The results of the separate studies of grub counts on the 30 animals used in cholinesterase investigations are shown in figure 1. The daily grub count of the 10 bolused animals had an increase from 38 to 43 grubs during the first 4 days followed by a sharp decrease in grubs during the next 54 days after which no grubs were found. The 10 animals treated with ET-57 in feed showed a sharp decrease from 33 grubs on the fourth day to 1 grub on the 13th day. Between the 30th and the 160th days after treatment, one live grub was observed. The 10 control

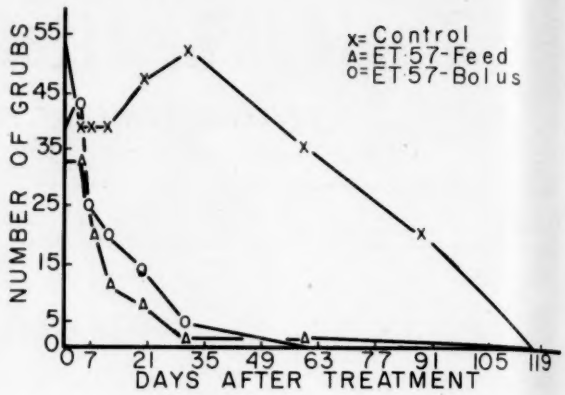

FIG. 1.—Average daily grub counts on 10 animals in each lot following the indicated treatment with ET-57.

Table 1.—Grub counts on combined lots of beef cattle treated with Dow ET-57.

TREATMENT	NUMBER OF ANIMALS	NUMBER OF GRUBS			
		Initial Count	Maximum Count	Number Increase	Average Increase per Head
Fort Hays Branch, Kansas Agricultural Experiment Station					
Bolus	118	354	413	59	0.50
Control	117	292	1,584	1,292	11.04
Juniata Ranch					
Bolus	80	260	290	30	0.38
In feed	80	187	207	20	0.25
Control	84	171	440	269	3.20

Table 2.—Average gain in weight (pounds) of 39 Hereford heifers of the Juniata herd treated for 1 day with 110 mg. of Dow ET-57/kg. body weight.[a]

TREATMENT	NO. OF ANIMALS	LOT 1[b]	LOT 2[c]	LOT 3[d]	LOT 4[e]
Control	15	408	456	428	477
ET-57-bolus	12	462	440	427	455
ET-57-feed	12	475	412	437	422

TREATMENT	AVERAGE OF ALL LOTS			
	Initial Weight	Final Weight	Gain	PER CENT INCREASE
Control	486	921	435	
ET-57-bolus	486	932	446	+2.5
ET-57-feed	481	923	442	+1.6

[a] Ten mg. of Stilbestrol per heifer per day were given during the 216 days of this experiment. All supplements fed as ½-inch pellets.
[b] One pound of 41% Gooch steer prema per day.
[c] Two pounds of 32% Gooch steer prema per day.
[d] Two pounds of 32% Gooch steer prema per day plus 70 mg. of auromycin per day.
[e] Two pounds of 32% Gooch steer prema per day plus 15 mg. of dynafac per day.

Table 3.—Average gain in weight (pounds) of 72 Hereford steers of the Juniata herd treated for 1 day with 110 mg. of Dow ET-57/kg. body weight.[a]

Treatment	No. of Animals	Lot 1[b]	Lot 2[c]	Lot 3[d]	Lot 4[e]	Lot 5[f]	Lot 6[g]
Control	25	509	554	506	537	465	478
ET-57-bolus	24	518	530	518	556	544	520
ET-57-feed	23	490	434	530	550	446	497

	Average of All Lots			
Treatment	Initial Weight	Final Weight	Gain	Per Cent Increase
Control	523	1031	508	
ET-57-bolus	522	1053	531	+4.5
ET-57-feed	526	1017	491	−3.3

[a] All supplements were fed for 225 days.
[b] Hand fed a complete meal fattening ration plus 30 mg. implant pellets of diethylstilbestrol; initially and after 100 days on feed.
[c] Self fed a complete meal fattening ration with 10 mg. of diethlstilbestrol per head per day.
[d] Hand fed a complete meal fattening ration with 10 mg. of diethylstilbestrol per head per day.
[e] Same as Lot 2 except ration in pellets as opposed to meal form.
[f] Same as Lot 4 except oats and barley replaced with wheat gray shorts.
[g] Same as Lot 5 except shorts substituted for oats and barley; 15% of milo in this ration was replaced with ground corn cobs.

animals showed a decrease from 54 grubs at time of treatment to 39 through the 4th, 6th and 12th days. Between 12 and 37 days after treatment, they showed a sharp increase to 52 grubs after which the number of grubs decreased steadily until at 116 days after treatment no grubs were observed.

Weight Studies.—Average weight gains (tables 2, 3, 4, and 5) of the cattle maintained on differing feed supplements and treated with ET-57 as a bolus and in feed were tabulated by the Western Star Mill, Salina, Kansas.

There were no consistent differences among weight

Table 4.—Average gain in weight (pounds) of 50 Hereford steers and heifers of the Juniata herd on wintering trials treated for 1 day with 110 mg. of Dow ET-57/kg. body weight.[a]

Treatment	No. of Animals	Lot 1[b]	Lot 2[c]	Lot 3[d]	Lot 4[e]	Lot 5[f]
Control	18	66	63	83	76	67
ET-57-bolus	16	59	79	97	48	84
ET-57-feed	16	45	48	70	50	58

	Average of All Lots			
Treatment	Initial Weight	Final Weight	Gain	Per Cent Increase
Control	360	431	71	
ET-57-bolus	367	440	73	+ 2.8
ET-57-feed	374	432	58	−18.0

[a] All lots received 1 pound of a 32% protein supplement containing various sources of phosphorus plus prairie hay free choice during the 180 days of this experiment.
[b] Phosphorus from colloidal clay and imported defluorinated rock phosphate.
[c] All phosphorus from colloidal clay.
[d] All phosphorus from dicalcium phosphate.
[e] All phosphorus from imported defluorinated rock phosphate.
[f] Control lot. All phosphorus from domestic defluorinated rock phosphate.

Table 5.—Average gain in weight (pounds) of 42 Hereford steers and heifers of the Juniata herd on wintering trials treated for 1 day with 110 mg. of Dow ET-57/kg. body weight.[a]

Treatment	No. of Animals	Lot 1[b]	Lot 2[c]	Lot 3[d]	Lot 4[e]	Lot 5[f]	Lot 6[g]	Lot 7[h]
Control	14	68	73	67	68	57	70	122
ET-57-bolus	14	42	82	22	98	83	32	145
ET-57-feed	14	100	82	87	90	120	62	105

	Average of All Lots			
Treatment	Initial Weight	Final Weight	Gain	Per Cent Increase
Control	424	499	75	
ET-57-bolus	414	486	72	− 4
ET-57-feed	405	497	92	+23

[a] All lots received prairie hay free choice during the 180 days of this experiment.
[b] One pound of a 32% cattle supplement.
[c] Same ration as Lot 1 except supplement contained 75 mg. of terramicin per pound.
[d] Same ration as Lot 1 except supplement contained 10 mg. of diethylstilbestrol per pound.
[e] One pound of solvent soybean oil meal, 44% protein.
[f] One pound of solvent cottonseed meal, 41% protein.
[g] One pound of a 32% protein supplement with a high molasses content, approximately 30% molasses.
[h] One pound of a 32% protein supplement plus 2 pounds of ground ear corn per head per day.

gains of the two treatments and the controls within each of the four lots. As a result, no statistical examination of the data was made since the differences appeared to be negligible for practical animal husbandry purposes. It was concluded that the few interesting but inconsistent overall differences were the result of normal variations in weights of the cattle and in the different types of supplement fed, and not due to the grub treatment alone.

The use of different types of feed supplements and different ET-57 treatments should be investigated in more detail.

Cholinesterase Studies.—The cholinesterase study on 30 animals from the Juniata experiment (fig. 2), showed that ET-57 produced a marked depression of enzyme activity.

The 10 animals treated with ET-57 boluses had a very

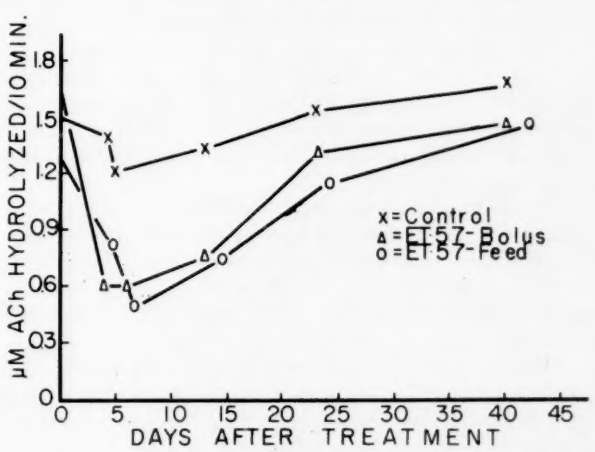

FIG. 2.—Average cholinesterase activity in whole blood withdrawn from 10 animals in each lot following the indicated treatments with ET-57.

sharp decrease at 4 days after treatment. They stayed at this level for 2 days and gradually returned to the enzyme activity level of the control animals by the 24th day.

The 10 animals given the treated feed showed a slower, but slightly greater, depression of enzyme activity. The recovery of the enzyme activity in these animals was essentially the same as in the bolused animals.

Toxicity.—No toxic symptoms were noted by the herdsman or by professional livestock men in any of the treated lots.

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Granulated Systemic Insecticides on Established Stands of Alfalfa for Control of the Spotted Alfalfa Aphid[1]

R. C. DOBSON[2,3]

ABSTRACT

The application of the systemic insecticides, Bayer 19639, Thimet and Am. Cyanamid 12008, on established stands of alfalfa was tested in granulated form for protection against the spotted alfalfa aphid, *Therioaphis maculata* (Buckton). Bayer 19639 at 1 pound of the active ingredient per acre was more effective than Thimet at the same rate during the "explosive" and relatively static high point of the infestation. Both materials were effective during the waning period of the population cycle in reducing the numbers of aphids to a non-economic level.

Am. Cyanamid 12008 was ineffective in controlling the spotted alfalfa aphid when applied in a granulated form to established stands.

The spotted alfalfa aphid, *Therioaphis maculata* (Buckton), is the primary pest of alfalfa in New Mexico. It has been found in 30 states and, during 1956 alone, caused losses of more than 42 million dollars to growers of this crop (Anon. 1957).

Procedures for the control of this aphid leave much to be desired. High winds may make it impossible to spray or dust at the proper time. Low temperatures reduce effectiveness of the insecticides used. Irrigation procedures may delay applications of the insecticides until after damage has occurred. Coverage of stubble or new regrowth of alfalfa plants has often been poor and has given poor results.

The above limitations and disadvantages prompted a search for new methods of application which might eliminate some of the objectionable features of spraying or dusting. Systemic insecticides, applied to the soil as granules, showed promise of avoiding some of the disadvantages of either spraying or dusting.

METHODS AND PROCEDURES.—The first experiment was designed as a pilot test, to determine the effectiveness of granulated formulations of systemic insecticides in reducing aphid populations on established stands.

New Mexico 11-1 alfalfa, an improved aphid-susceptible variety, was planted about September 15 in an irrigated field on the New Mexico A. & M. Experimental Dairy Farm. On October 16, 1956, granulated Thimet, Bayer 19639 (Disyston), and Bayer 23129 were applied (at approximately 1 pound of the active ingredient per acre) by broadcasting by hand over the surface of the plots. (An analysis made by the manufacturer in early November revealed only 0.2% concentration remaining of the original 2% Thimet material.) The field was irrigated by flooding within 6 hours following the application.

The soil was a heavy clay loam and at the time of application was cracked badly with the cracks extending 3 to 4 inches into the ground. It was thought that part of the granules would fall into the cracks and thereby be brought closer to the root systems of the plants. The alfalfa plants were 4 to 5 inches high and a heavy population of aphids was present. Considerable honeydew was showing on the leaves and considerable damage to the plants was apparent.

Readings on numbers of aphids present were taken by carefully placing a 4×6 inch index card alongside the plants, striking the plants sharply three times over the card, and then counting both winged and wingless aphids. Twenty counts per plot were taken at random in each plot.

Experiments 2, 3, 4 and 5 were planned to explore further the feasibility of controlling the spotted alfalfa aphid with systemic granulated insecticides on established alfalfa stands. Two fields of alfalfa (A. Lytton Farm, Fairacres, New Mexico) were used for this experiment, both of them very sandy. The first field consisted of a stand of seedling alfalfa planted in the early spring with some oats as a nurse crop. The second field was made up of a sparse stand of 5-year-old alfalfa which had considerable Ber-

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[3] Grateful acknowledgement is made to Robert Staten for his invaluable aid in the field work necessary for this paper.

muda grass interspersed throughout. Each treated plot consisted of one-tenth of an acre running from border to border in each test area. Fifty-foot buffer areas were laid out between the treated plots in the first two experiments, since it was thought that, under irrigation by flooding, the granulated material might be washed a considerable distance down the test area. This did not prove to be the case, so these buffer areas were eliminated from experiments 4 and 5.

In experiment 2, the materials (Bayer 19639 and Thimet) were broadcast by hand over the surface immediately before irrigation. Thimet as 2% granulated material was applied at the rate of 1 pound of active ingredient per acre. Bayer 19639, in a similar formulation, was applied to two plots, one at the rate of 1 pound active ingredient per acre and the other at a half-pound. The hay was 4 to 6 inches high at the time of treatment. Counts of the aphid infestation were made in the same manner as in experiment 1 with the exception that only 10 counts were made per plot. Insecticides were applied on April 25, 1957, and the first count taken on April 29.

Experiment 3 was set up in the same manner as experiment 2. The same materials were used but they were applied on May 4 and the first counts were taken on May 6. The alfalfa had been cut once and the hay removed. It had not started to grow so the stubble was about 2 inches high at time of application. The materials were applied about noon and water reached the plots about 7 hours later. The alfalfa was about 4 inches high at the time of first counting.

Experiment 4 was essentially a repetition of experiments 2 and 3. The plots were located in new areas in the spring-planted alfalfa so that they could not be contaminated by previous applications. One additional material, Am. Cyanamid 12008, was incorporated into this experiments as a 2% granulated material and was applied at 1 pound of the active ingredient per acre. The materials were all applied with a Cyclone Seeder set to apply the proper amount of material per plot. The application of materials was made on June 18, 1957 and watered immediately. The first readings were taken on June 19 when the alfalfa was about 6 inches high due to a shower of rain which followed cutting and preceded the application of the insecticide.

Experiment 5 was conducted in the same manner as experiment 4. It was located in the 5-year-old alfalfa field in new areas in order to prevent contamination. The application was made on the same day as experiment 4, June 18. It was watered immediately and the first count was taken on June 19. The plants were about 6 inches high since these plots, too, had received a shower of rain before the application of the insecticides.

RESULTS.—*Experiment 1.*—Due to the heavy clay texture of the soil, it was not possible to get into the field until 8 days after irrigation. On October 24, 1956, counts of winged and wingless aphids were taken (table 1). At this time, the untreated check and the Thimet-treated plots were badly covered with honeydew and mold was appearing on the ground. The plots treated with Bayer 19639 and Bayer 23129 had no honeydew and no apparent damage to the plants. Most of the wingless aphids in the plot treated with Bayer 19639 had wing pads, while this condition was not true of the aphids in the other plots.

Table 1.—Aphid counts on treated plots. Oct. 24, 1956.

TREATMENT[a]	NUMBER OF APHIDS[b]	
	Winged	Wingless
Bayer 19639	14	33
Bayer 23129	23	155
Thimet	34	524
Check	17	681

[a] All materials were supposed to contain 1 pound actual toxicant per 50 pounds of material. The Thimet tested 0.2% actual toxicant in November 1956. Application was made at the rate of 50 pounds per acre.
[b] Total aphids per 20 samples.

A hard freeze on the night of October 26 reduced the populations in all plots to the point where further readings were worthless.

Experiment 2.—Population counts on the various plots of experiment 2 were made on the dates shown in figure 1. From the data obtained, it is evident that the population was in the "explosive" stage for the duration of the experiment It is also apparent that both materials were effective in reducing and holding the populations down for 11 days after application. It is evident that the Bayer 19639, either at the 1-pound or half-pound rate, continued to hold the populations in check longer than the Thimet. The alfalfa was harvested on May 17 so no further readings were taken. The hay made from the plots treated with

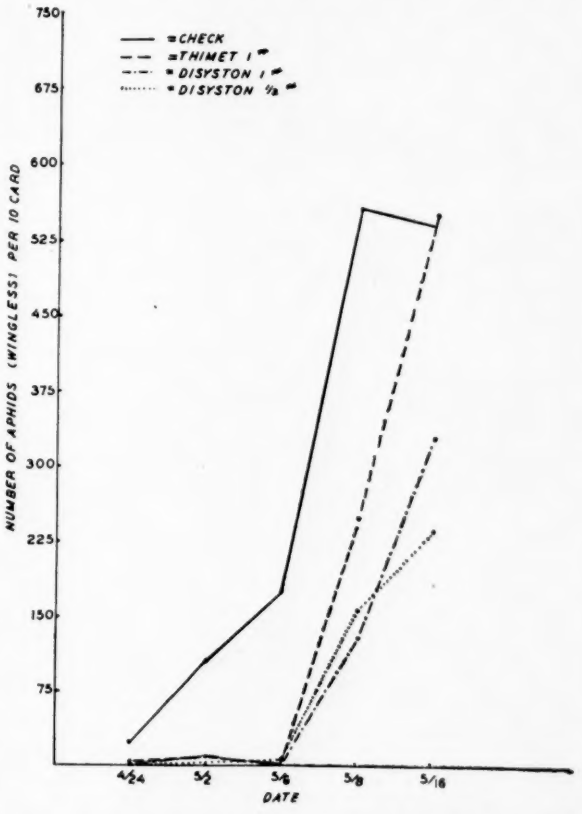

FIG. 1.—Results of granulated systemic insecticide applied April 24, 1957 to an established stand of young alfalfa during the "explosive phase" of an infestation. Disyston is Bayer 19639.

Bayer 19639 was in very good condition with very little honeydew and no sooty mold beneath the plants. The untreated check block was severely injured and was sticky, with sooty mold halfway up the stems. Hay made from the Thimet-treated plot had considerable honeydew and yellowing of the lower leaves. No sooty mold was seen on this hay.

Experiment 3.—The data obtained in this experiment discloses some important points in the control of spotted alfalfa aphid (fig. 2). A very high population of aphids was present in this field when the insecticides were applied. The population in the untreated check decreased with each reading because the tremendous population reduced not only the growth of the plants but also their quality, so the aphids were unable to maintain themselves in large numbers. At the end of the growing period, the plants in the untreated check had produced virtually no growth and the stems left standing were denuded of leaves.

The insecticides, Bayer 19639 and Thimet, at the 1-pound rate were equally effective in holding these populations down for 11 days, but after this period, they began to lose their effectiveness. The population on plots treated with Thimet expanded very rapidly while the increase in the population on plots treated with Bayer 19639 was slower.

The half-pound rate of Bayer 19639 was partially effective but was not heavy enough to bring the population down to the noninjurious level. This can be seen from the fact that the population started to go up 6 days after application, went to a high level 12 days after application and then, when the alfalfa was reduced in attractiveness, started downward in a pattern similar to that on the untreated check. At cutting time, June 1, the hay made from the plot treated with Thimet was yellowish at the top and black on the bottom. The leaves were very gummy and there were very few leaves remaining on the stems. The hay made from the plot treated with Bayer 19639 at the half-pound per acre rate was very gummy and showed obvious symptoms of injury. Hay made from the plot treated with 1 pound of Bayer 19639 was just starting to show honeydew droplets at cutting time. Virtually no hay was harvested from the untreated check plot.

Experiment 4.—The application of Thimet and Bayer 19639 again proved very effective in reducing aphid populations. After 5 days, the aphid counts on the plots where these materials had been applied, were very low (fig. 3). Am. Cyanamid 12008 was not effective in reducing the population of aphids. The number of aphids in the untreated check plot also dropped during the first 5 days. Immediately thereafter the populations in the untreated check and in the plot treated with Am. Cyanamid 12008 began to rise. The plots treated with Bayer 19639 or Thimet on the other hand, remained virtually clear of aphids. By the end of the experiment on June 26, when the hay was harvested, the check and the plot treated with Am. Cyanamid 12008 bore considerable honeydew

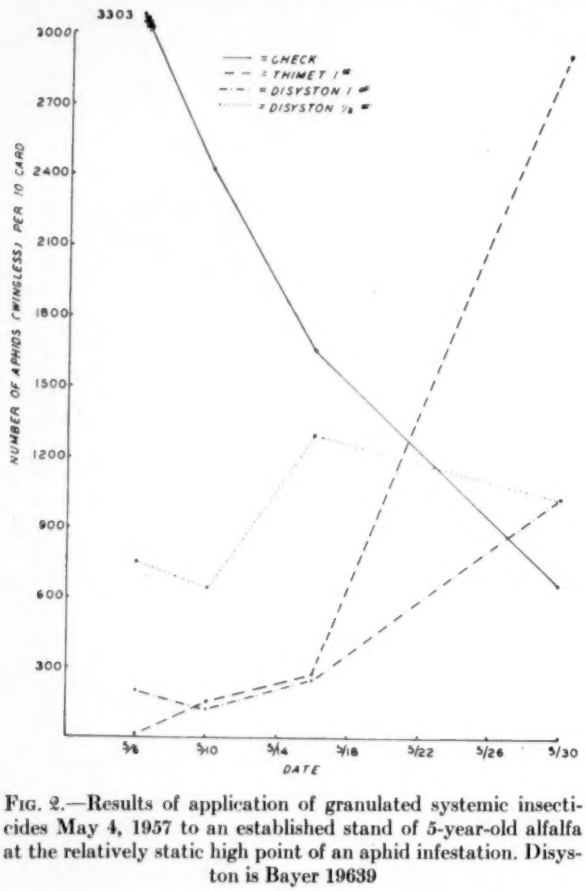

FIG. 2.—Results of application of granulated systemic insecticides May 4, 1957 to an established stand of 5-year-old alfalfa at the relatively static high point of an aphid infestation. Disyston is Bayer 19639

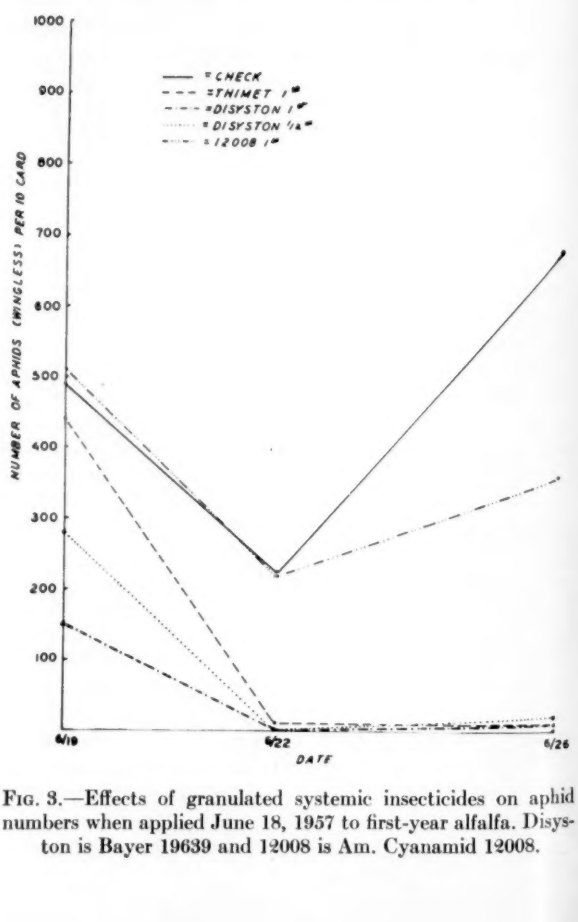

FIG. 3.—Effects of granulated systemic insecticides on aphid numbers when applied June 18, 1957 to first-year alfalfa. Disyston is Bayer 19639 and 12008 is Am. Cyanamid 12008.

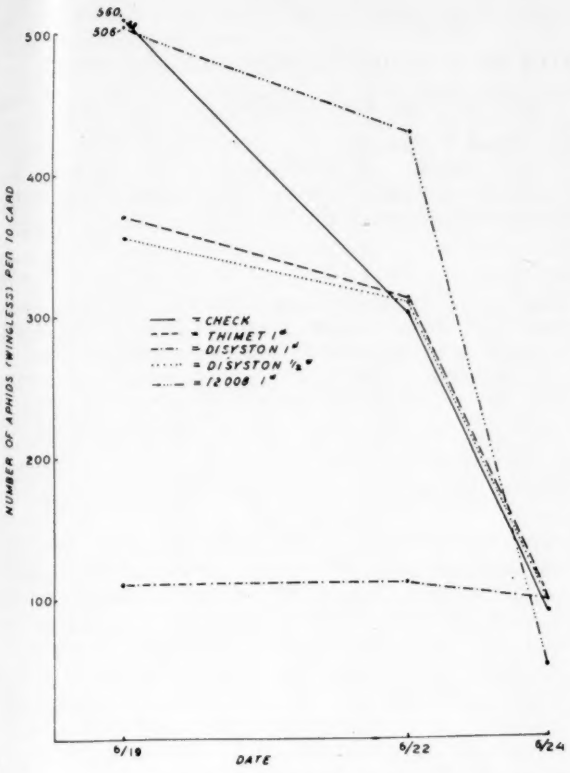

FIG. 4.—Effects of granulated systemic insecticides when applied June 18, 1957 to 5-year-old alfalfa during the "declining phase" of an aphid infestation. Disyston is Bayer 19639 and 12008 is Am. Cyanamid 12008.

accompanied by some shedding of leaves in the lower third of the plants. No injury to the alfalfa treated with Bayer 19639 or Thimet was evident. As may be seen from figure 3, the aphid population was relatively static during this period.

Experiment 5.—On June 18, when this experiment was started, the population of aphids was declining. This may be readily seen in figure 4. The population of aphids in the untreated check and in the plot treated with Am. Cyanamid 12008 started out high but by the end of the test period was low. Plots receiving the half-pound of Bayer 19639 or Thimet showed much the same pattern but started lower at the beginning since these dosages were partially effective in the control of the aphid. The plot treated with 1 pound of Bayer 19639 had a fairly low number of aphids in the beginning of the experiment in which the population remained relatively static throughout. Alfalfa hay harvested from these plots on June 25 was virtually undamaged.

CONCLUSIONS.—Application of granulated systemic insecticides to established stands of alfalfa showed promise in controlling the spotted alfalfa aphid. This method of application eliminates, or at least ameliorates, some of the hazards inherent in spraying or dusting. Bayer 19639 at the rate of 1 pound of active ingredient per acre was effective in controlling this insect for at least 11 days and continued to partially control it for some time thereafter. At half-pound per acre it was only partially effective. Thimet at the rate of 1 pound of active ingredient per acre was also effective in controlling this aphid. It gave protection about 11 days after which its toxicity was gone. Am. Cyanamid 12008 was virtually ineffective in reducing populations of this pest.

It was also evident that Bayer 19639 at 1 pound per acre is more effective than Thimet in controlling the spotted alfalfa aphid during the "explosive period" of an infestation and also at the relatively static high point of the infestation. During the waning period of the population cycles, Thimet and Bayer 19639 at 1 or 0.5 pound per acre reduced the population to a non-economic level.

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First Records of Two Pine Sawflies in Michigan[1]

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In the course of a study of the geographic distribution of the Swaine jack-pine sawfly, *Neodiprion swainei* Middleton, it became apparent that there was no record of its presence in the United States of America except from Wisconsin (Benjamin & Underwood 1953, Ross 1955). From knowledge of the distribution of this species in Ontario and taking into account the prevalence of jack pine, *Pinus banksiana* Lamb., in the Lake States, it seemed likely that the sawfly occurs throughout the range of jack pine in Michigan, Wisconsin, and Minnesota. On August 21, 1957, we had the opportunity to examine jack pine at 15 localities in the eastern end of Michigan's Upper Peninsula and found colonies of *N. swainei* larvae in Chippewa, Luce, and Mackinac counties. The larvae were mainly fourth instar and were typical for the species. In each instance the old egg cluster was found near the feeding site and the oviposition pattern provided confirmation of our identification.

Three unusual *Neodiprion* larvae were found feeding on jack pine east of Caffey in Mackinac County. The data available lead us to believe that the larvae were those of *Neodiprion nigroscutum* Middleton, one of the more poorly-known species in the genus. A single female adult has been reared and agrees reasonably well with the original description of *N. nigroscutum* by Middleton (1933) and with recent keys to the genus (Peck 1943, Ross 1955).

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Carbamoyl Alkyl Phosphorodithioates as Chemotherapeutic Agents: Screening by Aedicidal Properties in Laboratory Mammals, Lambs and Calves[1]

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ABSTRACT

Procedures are given for the use of a mouse-mosquito (*Aedes aegypti* (L.)) test, and supplementary tests in other mammals, in screening and evaluating compounds for chemotherapeutic activity. Nine of thirteen known insecticides given orally to mice killed mosquitoes feeding on them, including six out of seven compounds known to be effective against cattle grubs. The most active of 19 carbamoyl alkyl phophorodithioates tested was O,O,-dimethyl-S-(N-methylcarbamoylmethyl) phosphorodithioate (Am. Cyanamid 12,880). This had an ED_{50} in mice, lambs and calves of about 6 mg./kg., with a chemotherapeutic index (LD_{50}/ED_{50}) of about 25 in mice. In lambs and calves, about seven times the ED_{50} for mosquitoes produced marked toxic symptoms. Structure-activity relationships in the carbamoyl alkyl phosphorodithioates indicated a marked superiority of O,O,-dimethyl compounds over corresponding O,O,-diethyl analogs. The few higher O,O,-alkyls tested were uninteresting. In the O,O-dimethyl series, the unsubstituted carbamoyl group was less than one-twentieth as active as the methyl carbamoyl.

Earlier attempts to attack arthropod ecto- and endoparasites of birds and mammals by the oral or parenteral administration of chemical compounds to their hosts are reviewed by McGregor *et al.* (1954, 1955), McGregor & Bushland (1956), and Radeleff & Woodward (1956). Such an approach seeks chemotherapeutic agents which are relatively nontoxic to the host, but which will be absorbed and distributed to the sites where arthropod parasites are feeding.

Although other types of chemicals have been reported to possess chemotherapeutic activity against arthropods (see reviews cited above), the chlorinated hydrocarbons and the organic phosphorus compounds have been studied most intensively. Many studies were in cattle infected with grubs (*Hypoderma lineatum* (De Vill.) or *H. bovis* (L.)), since these are among the most important livestock pests in the United States which are not easily and adequately controlled with externally applied insecticides. Several chlorinated hydrocarbons are not considered promising, although effective against grubs when administered internally, because they leave undesirable residues in the fat of treated animals and are ineffective against the first instar larvae (Radeleff & Woodward 1956). More encouraging results have recently been obtained with organic phosphorus compounds (McGregor *et al.* 1954, Roth & Eddy 1957, McGregor & Bushland 1957, Radeleff & Woodward 1957, Adkins 1957, Anonymous 1957).

Various host-parasite relationships have been used for screening and evaluating this type of chemotherapeutic activity in the laboratory. Rabbits, guinea pigs, or mice are used commonly as mammalian hosts, and screwworms, deer flies, stable flies, bedbugs, lice, ticks, or mosquitoes as the test arthropods (Lindquist *et al.* 1944, 1953, de Meillen 1946, Garnham 1948, Knipling *et al.* 1948, McGregor & Bushland 1956).

In the present studies, the initial screening, and preliminary evaluations of active compounds, were conducted with mice and *Aedes aegypti* mosquitoes. *A. aegypti* and guinea pigs, rats, sheep, and cattle were used for further evaluation in mammals, previous to testing in grub-infected cattle, which will be reported subsequently.

The usefulness of *A. aegypti* as test arthropods for initial screening and evaluation was indicated early in this work. A fair degree of correlation occurred between the aedicidal activity in mice with the activity against cattle grubs reported by other investigators for several chlorinated hydrocarbons (lindane, aldrin, and dieldrin) and two organic phosphorus compounds (Dipterex and Chlorthion). This correlation was substantiated further with the new type of chemotherapeutically active phosphorodithioate reported in this paper.

Phosphorodithioate compounds for testing were obtained from the Agricultural Chemicals Section, Research Division, American Cyanamid Co., Stamford, Connecticut. The cooperation of the following is acknowledged gratefully: Doctors Elton Clark and Frank Stark for selecting compounds for testing, and Doctors Gerald Johnson and Richard Young and their colleagues for the synthesis of compounds. We are also indebted to Wyeth Wallace, John Emro, Joseph Entwistle, John Pankavich and Doctor Ralph Thorson, our associates in the Parasitic Chemotherapy Department, for assistance in the sheep and cattle tests.

METHODS.—*The mouse-mosquito test.*—Ova of *A. aegypti* were obtained initially from the Laboratory of Tropical Diseases, National Institutes of Health, Bethesda, Md. Conventional methods were used for rearing (Trembly 1955). Preliminary acute toxicity data on mice or rats of the test compounds from the Toxicology Department, American Cyanamid Co., Stamford, Conn., were supplemented by tests in three mice each at single oral doses of 10, 50, and 250 mg./kg., and an observation period of at least 4 hours after treatment. In general, compounds were tested first for activity at single oral doses of 250 mg./kg., or, when this was not tolerated, at one-fifth the lethal dose (or the dose producing marked symptoms). Compounds lethal at 10 mg./kg. were tested first at 1 mg./kg. Occasionally, test doses were used which were lethal to mice shortly after 1 hour. Compounds of special interest were titrated from the 90 to 100% level of activity and/or toxicity to the no-effect level, usually in two-fold

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decrements. Thirteen known insecticides were tested at maximum tolerated doses if inactive.

The mosquitoes were used usually within 1 week after pupation, and were maintained on 5% dextrose solutions until the blood meal and thereafter. Three mice per dose were treated with the test compounds, and about 10 female mosquitoes were applied to each of one or two mice 1 hour after dosage. The mice were shaved ventrally, treated with Nembutal ® pentobarbital sodium, Abbott about 45 minutes after dosage (90 mg./kg. intraperitoneally in 0.5 cc. of water), and placed ventrally over the bobinette tops of isolation jars containing the mosquitoes. From 30 to 45 minutes were allowed for feeding. Additional feedings after the 1-hour interval rarely changed the initial evaluation of activity. The number of dead mosquitoes was recorded at 24, 48, and 72 hours after each feeding. Generally, with the phosphorodithioates, most deaths occurred within 24 hours after feeding at levels close to the ED_{50} (the dose which killed approximately 50% of the mosquitoes). With lower doses, deaths were delayed occasionally, although early symptoms of toxic effects (*i.e.* tremors, incoördination) occurred frequently. In each test, about 20 control mosquitoes were fed on two untreated, anesthetized mice at times comparable to those fed on treated mice. Only mosquitoes with blood meals were observed for toxicity. These were separated from unfed mosquitoes by chilling for 15 to 30 minutes (jars containing mosquitoes were placed in ice water), and isolating those with distended abdomens while they were quiescent. Uneventful recovery from chilling occurred promptly.

Aedicidal activity in large rodents and ruminants.—If the degree of activity in mice was promising, limited tests were made in anesthetized rats and/or guinea pigs and non-anesthetized lambs and calves. In general, the doses selected were comparable to those used in mice. Both parenteral and oral administration were employed, and methods for applying and observing mosquitoes were similar to those used for mice with appropriate modifications for the larger size of the hosts.

Toxicity.—Toxicity studies in mice and guinea pigs were generally limited (see "Methods," first paragraph) and included only rough approximations of the maximum tolerated dose or of the LD_{50} dose with our test conditions. Toxicity and activity studies were combined in many of the lamb and calf tests and will be described in a later publication.

MATERIALS.—All of the commercial insecticides listed in tables 1 and 2 were technical grade products of unknown purity. Compounds XII through XIX in table 3 were ordinary technical materials prepared according to the procedure of Hoegberg & Cassaday (1951). Compounds I through XI were analytically pure compounds prepared by modifications of the Hoegberg and Cassaday procedure (R. W. Young *et al.* to be published). Comparisons among compounds I through XI therefore may be made with considerable confidence.

RESULTS.—*Lindane and untreated-control standards.*—The data from mice treated with lindane as compared with untreated controls (table 1) illustrate several points which are applicable to the screening and evaluation tests in general. Some mortality occurred in mosquitoes fed on

Table 1.—Mortality of *Aedes aegypti* fed on untreated mice or on mice treated with a single oral dose of lindane.

Lindane (Mg./Kg.)	No. Experiments	No. *Aedes* Applied[a] Hours After Mice Dosed: 1	No. *Aedes* Applied[a] Hours After Mice Dosed: 4	Per Cent Mortality After Feeding: 24 Hours, Hours After Dosage of Mice: 1	24 Hours: 4	72 Hours: 1	72 Hours: 4
None	53[b]	725 (72)	512 (65)	0.8	1	8[d]	8
None	29[c]	343 (64)	333 (63)	0.3	0	9	9
12.5	4	92 (77)	101 (86)	1	0.9	7	9
25	16	207 (72)	196 (71)	1	3	23	44
50	13	169 (64)	168 (67)	8	5	60	67
100	15	141 (54)	130 (52)	24	20	87	86

[a] Percentages that fed are given in parentheses.
[b] Including all untreated control groups where observations were made on mosquitoes 72 hours after blood meals. Only 43 groups at the 4-hour feeding period.
[c] Including only the untreated controls used in the lindane experiments. Only 28 groups at the 4-hour feeding period.
[d] 50% or more mosquitoes died in only one of 53 test groups; lindane data for this test not included in the table.

Table 2.—Comparison of the oral chemotherapeutic activity in mice of various insecticides against *Aedes aegypti*.

Compound	Per Cent Mortality[a] of *Aedes aegypti* Fed on Mice 1 and 4 Hours After Oral Dosage at Mg./Kg. Indicated: >500 mg./kg.	>500 % kill	500	250	100	50	25	<25 mg./kg.	<25 % kill
Lindane	—	—	—		87 (271)	64 (337)	41 (511)	12.5:	8 (193)
Malathion	1,500:	71 (18)[b]	4 (27)		—				
DDT	1,000:	37 (19)[b]	—		—				
Chlorthion	750:	40 (33)	50 (14)[b]		0 (34)				
Methoxychlor	5,000:	0 (12)	—		—				
Chlordane	—	—	78 (18)[b]	62 (16)[b]	25 (19)[b]	0 (33)	—		
Aldrin					90 (40)	52 (73)	49 (68)	12.5:	0 (35)
Dieldrin					60 (53)	6 (38)	0 (30)		
Dipterex					87 (15)	87 (31)	6 (72)		
Diazinon					9 (22)	11 (28)	6 (36)		
Demeton							12 (34)	10:	15 (33)
Parathion								10:	4 (27)
Dow ET-57			97 (38)	89 (35)	77 (39)[c]	46 (37)		20:	12 (34)

[a] Pooled except where significant differences occurred between 1- and 4-hour feedings. Numbers that fed are shown in parenthesis.
[b] 1-hour feedings only; significantly less activity at 4-hour feeding.
[c] 125 mg./kg.

Table 3.—Structure-activity relationships in the mouse-mosquito test of carbamoyl alkyl phosphates.

$(RO)_2—\overset{\overset{S}{\|}}{P}—S—CH_2—CONHR'$

Compound No.	R=	R'=	ED_{50}-*Aedes*—Mg./Kg. Calculated[a]	ED_{50}-*Aedes*—Mg./Kg. Estimated[b]	LD_{50}-Mice—Mg./Kg. Calculated[a]	LD_{50}-Mice—Mg./Kg. Estimated[c]
I	CH_3	H	135 (123–149)		—	>250
II	CH_3	CH_3	5.7 (5.1–6.3)		140 (124–157)	
III	CH_3	C_2H_5	44 (35–55)		350 (250–490)	
IV	CH_3	C_3H_7	—	>50<75	550 (440–688)	
V	CH_3	C_3H_7 *iso*	44 (34–39)		370 (264–518)	
VI	CH_3	C_4H_9	35 (29–42)		—	150
VII	CH_3	C_4H_9 *iso*	74 (68–82)		180 (150–216)	—
VIII	CH_3	C_4H_9 *tert.*	30 (27–34)		1,050 (778–1,417)	—
IX	CH_3	$CH_2CH=CH_2$		>250		>250
X	CH_3			>250		>250
XI	CH_3			>250		>250
XII	C_2H_5	H		35		10–20
XIII	C_2H_5	CH_3		> 50		5–10
XIV	C_2H_5	C_2H_5		> 50		50
XV	C_2H_5	C_3H_7 *iso*		> 50		40–50
XVI	C_3H_7	H		>100		<100
XVII	C_3H_7	CH_3		> 50		< 50
XVIII	C_3H_7	C_2H_5		> 50		<250
XIX	C_3H_7 *iso*	CH_3		>250		<250

[a] By the Litchfield-Wilcoxon Method (1949). (95% confidence limits.)
[b] < indicates greater than 50% aëdicidal activity at the dose indicated.
> indicates less than 50% aëdicidal activity at the dose indicated.
[c] < indicates greater than 50% mortality in mice at the dose indicated.
> indicates less than 50% mortality in mice at the dose indicated.

untreated mice during a 3-day interval following feeding. The total number of deaths 24 hours after feeding was negligible. At the 72-hour interval, the deaths were 8% of the total in 53 test groups with more than three mosquitoes, representing 47 different trials. However, in 29 of the 53 groups (55%), with a mean of nine mosquitoes feeding at the 1-hour period, no mosquitoes died 72 hours after feeding on untreated mice. In 12 groups (23%), only one mosquito died, and in the remaining 12 groups the mortality varied from two to seven out of a mean of 16 mosquitoes. In only four of 53 control groups did mortality exceed 33%, and in only one group did it exceed 50% (7 out of 13 mosquitoes dead). Similar results were obtained on analysis of the 4-hour feeding period in 43 groups.

Since the death of more than one mosquito out of 10 fed on untreated mice occurred relatively infrequently, a mortality of 50% or more of the mosquitoes fed on treated mice was considered suggestive of activity, providing that the mortality in the controls of the specific test followed the usual pattern. The latter qualification is important. All data from very occasional tests were discarded when a large proportion of the control mosquitoes died from unknown causes on the first or second day after feeding.

From 20 to 30% mortality of mosquitoes was considered border-line activity, even though the untreated controls suffered 0 to 10% mortality. Confirmation was attempted only if the compound had special structural interest or a possible margin of safety.

Lindane was tested repeatedly as a possible drug standard for this test procedure (table 1). Single oral doses of more than 25 mg/kg. and less than 50 mg./kg. of lindane were required to kill within 72 hours 50% of the mosquitoes fed on treated mice both 1 and 4 hours after dosage. The only significant difference between the results at the 1- and 4-hour feeding was with a dose of 25 mg./kg. ($p=0.01$). The 24-hour observation period gave little indication of the lethality of lindane to mosquitoes, with the possible exception of 100 mg./kg. However, characteristic symptoms were observed in mosquitoes fed on lindane-treated mice within a few hours, and continued until death. At the 100 mg./kg. dose of lindane, the percentage of applied mosquitoes that fed was somewhat less than with the lower doses and with the untreated mice ($p=<0.05$), suggesting a possible repellent effect.

Results with lindane were somewhat variable in different tests, but estimates of the ED_{50} were generally within two-fold. Lindane may not be the most suitable standard drug for comparison with phosphorodithioates. Most of the latter showed a sharp drop in insecticidal activity by this assay between the 1- and 4-hour feedings (i.e. blood concentrations are not persistent), whereas lindane was as active at 4 and 24 hours as at earlier intervals after feeding.

Effects of 12 other known insecticides.—Lindane, aldrin, dieldrin, Dipterex and Dow ET-57 were active at 25 to 100 mg./kg.; chlordane and chlorthion at 250 to 500 mg./kg.; and malathion and DDT at 1,000 to 1,500 mg./kg. (table 2). Methoxychlor, Diazinon, demeton and parathion were inactive at maximum tolerated doses. Of the seven which have known chemotherapeutic activity against cattle grubs (lindane, Chlorthion, aldrin, dieldrin, Dipterex, Diazinon and Dow ET-57), only Diazinon was

inactive against mosquitoes. The relative activity of lindane, aldrin, and Dipterex against mosquitoes fed on treated mice was roughly similar to that reported for cattle grubs (McGregor *et al.* 1954, 1955).

Aedicidal activities of carbamoyl alkyl phosphorodithioates.—Some 150 organic phosphorus compounds, representing diverse structural types and insecticidal activity have been tested. The most interesting were of the type:

$$(RO)_2\text{—}\overset{\overset{S}{|}}{P}\text{—}S\text{—}CH_2CONHR'.$$

Where R = CH_3 in table 3, eight of the 11 compounds listed were active in the mouse-mosquito test, with ED_{50}'s ranging from 6 to 135 mg./kg. in a single oral dose. Am. Cyanamid 12,880 (compound number II in tables 3 and 4): O,O-dimethyl-S-(N-methyl-carbamoylmethyl) phosphorodithioate, for which the common name, dimethoate, is used in this paper, was the most active and, excepting compound VIII, had the widest margin of safety between aedicidal doses and doses toxic to mice. When R was ethyl, propyl or isopropyl the compounds were generally inactive at tolerated doses or were more toxic than the corresponding dimethyl compounds.

Aedicidal activity of dimethoate in large rodents and ruminants.—Further evaluations of dimethoate were made in rats, guinea pigs, lambs, and calves, with *Aedes aegypti* as the test arthropod. A summary of pooled data from several tests in each host, following single oral doses, is given in table 5. At doses of approximately 10 to 15 mg./kg., or higher, activity was remarkably similar in all five mammalian hosts. With 5 to 6 mg./kg., the response was more variable, with highly significant lower activities in guinea pigs and lambs as compared to rats and mice.

Seven other active carbamoyl alkyl phosphorodithioates listed in table 3 were also tested in guinea pigs. Only one, compound number XIII (table 3), was more active (at least 10-fold) in the guinea pig than the mouse; three were equally active; one was less active, and two were inactive at maximum tolerated doses. The high toxicity of compound number XIII in mice, guinea pig activity only at 4 mg./kg., plus a lethal effect in one lamb at 10 mg./kg., discouraged further evaluations.

Table 5.—Chemotherapeutic activity of dimethoate in various mammals against *Aedes aegypti*.

Host	Per Cent Mortality in 72 Hours[a] (1 Hour After Being Given Mg./Kg. Oral Dosage Indicated)			
	1–3	5–6	10–15	25
Mouse	20 (59)	70 (57)	96 (59)	100 (59)
Rat	40 (47)	90 (30)	95 (20)	
Guinea pig	7 (28)	12 (60)	93 (73)	90 (72)
Lamb		25 (40)	90 (69)	87 (49)
Calf	18 (67)	50 (28)	76 (106)	96 (23)

[a] Numbers of *Aedes aegypti* that fed are given in parentheses.

Duration of the aedicidal activity of dimethoate in livestock.—The aedicidal activity of dimethoate diminished rapidly at some period prior to 24 hours after dosage (table 6). Very few mosquitoes died when fed at the 24-hour interval as compared with a high percentage mortality at 1 and 4 hours, and in the one calf tested at 8 hours. This suggests rapid elimination of the compound or its active metabolites from the blood stream.

Effects of different routes of administration of dimethoate.—Aedicidal concentrations in the blood of various mammals were obtained within 1 hour after the administration of dimethoate at 10 to 25 mg./kg. orally, intramuscularly, or subcutaneously. The possible superiority of a given route in the *Aedes* test has not been established. In calves, the oral route was effective with the compound given by drench, bolus, or gelatin capsule; the intramuscular route was effective in a water and acetone solution or in a 50%

Table 4.—Titrations in the mouse-mosquito test of compounds listed in table 3.

Compound No.	Per Cent Mortality in 72 Hours After Feeding on Mice[a] (1 Hour After Being Given Mg./Kg. Oral Dosage Indicated)							
	1–3	5–6	10–15	20–30	40–50	100	200	250
I	—	—	—	—	10 (19)	30 (46)	60 (48)	100 (8)
II	12 (111)	58 (84)	88 (149)	99 (81)	—	—		—
III		0 (38)	18 (39)	57 (40)	91 (35)	—		—
IV		—	0 (16)	0 (14)	7 (14)	15 (26)		97 (28)
V		0 (40)	46 (39)	55 (40)	83 (42)	—		—
VI			5 (37)	16 (31)	68 (31)	100 (30)		—
VII				10 (20)	28 (36)	71 (28)		85 (14)
VIII				13 (56)	94 (53)	100 (18)		—
IX				—	—	0 (16)		40 (40)
X								5 (38)
XI								0 (15)
XII	—	—	9 (45)	38 (27)	86 (7)			—
XIII			—	—	20 (10)			
XIV			0 (20)	15 (39)	32 (40)			
XV			5 (20)	5 (57)	28 (42)			
XVI			—	—	—	20 (20)		
XVII		5 (38)	8 (12)	5 (18)[b]	12 (16)[b]			
XVIII		—	—	—	0 (20)			
XIX		—	—	—	—			0 (14)

[a] Numbers of *Aedes aegypti* that fed are given in parentheses.
[b] At least one mouse survived for the 1-hr. feeding period.

Table 6.—Duration of the chemotherapeutic activity of a single oral dose of dimethoate in calves and lambs.

Animal No.	Dosage (Mg./Kg.)	Per Cent Mortality in 72 Hours[a]			
		Hours After Treatment			
		1	4	8	24
Calf 14	40	100 (25)	100 (32)		9 (23)
Calf 200	25	96 (23)	100 (25)		5 (22)
Calf 21	15	100 (25)		97 (26)	
Calf 300	10	32 (25)	89 (27)		4 (27)
Lamb 52	25	75 (24)	67 (21)		4 (25)
Lamb 53	10	85 (23)	70 (17)		9 (22)

[a] Numbers of *Aedes aegypti* that fed are given in parentheses.

diethylsuccinate solution (dimethoate gives 2 to 3% water solutions). Peanut oil was used as the vehicle for intramuscular and subcutaneous administration in sheep.

Effects against lice on mice.—Preliminary data indicate a partial effect with single oral doses of 12.5 mg./kg. of dimethoate against natural infections with *Polyplax sp.* on mice. In four out of five treated mice, from 50 to 80% of the lice were killed and dropped off the host within 24 hours, compared with 8 to 14% in four untreated controls. Total numbers of lice per mouse ranged from 85 to 2,400. No significant effect was obtained in one treated animal with an unusually severe infection.

Toxicity data.—Compounds less toxic than dimethoate were also less aedicidal and usually not superior in the margins of safety in mice (table 3). Thus, compound numbers III, IV, V, VI, and VII, the related ethyl, *n*-propyl, isopropyl, butyl and isobutyl carbamoyl analogs had margins of less than 10-fold. Similarly estimated margins with dimethoate (compound number II, table 3) were about 25-fold in the mouse-mosquito test. Compound number VIII, the tertiary butyl analog, had a margin of 35-fold.

Acute toxicity data with dimethoate in lambs and calves will be presented in a subsequent paper. Severe, reversible toxic symptoms appeared with 40 to 60 mg./kg. orally, and lethal effects with 80 to 100 mg./kg. The margin between aedicidal doses (5 to 10 mg/kg.) and toxic mammalian doses is accordingly appreciable in these ruminants, but narrower than in the mouse.

Discussion.—In the current studies, the decision to use the mouse-mosquito test for screening and preliminary evaluations was based largely upon the small size of the mammalian host, permitting tests with very small samples, and the ease with which mosquitoes can be raised in large numbers. Previous studies had also demonstrated that lindane was chemotherapeutically active against mosquitoes when administered to small mammals (deMeillon 1946, Garnham 1947, Knipling *et al.*, 1948, Lindquist *et al.* 1953) as well as *Hypoderma* in cattle (McGregor *et al.* 1955). As with other large-scale screening tests, using substitute hosts and parasites, there is no assurance that new compounds selected by this test will invariably give some degree of activity against grubs in cattle or against other arthropods in cattle or other hosts where the chemotherapeutic attack would be desirable. The comparative results, however, have been encouraging thus far, both with older compounds having known chemotherapeutic activity and with the newer types reported here.

Our observations indicate the desirability of holding the *Aedes* 72 hours after they have fed on treated animals. The active carbamoyl phosphorodithioates usually exerted their effects rapidly, and in many cases the majority of mosquitoes died within 24 hours after feeding. Dow ET-57 and lindane were less rapidly lethal, although definite symptoms could be observed frequently in the mosquitoes shortly after feeding, and over a 3-day period these mosquitoes died. Dimethoate was 10 times more active than Dow ET-57 and five times more active than lindane in the mouse-mosquito test.

One variable which was not controllable was the amount of blood consumed by individual mosquitoes. Although all mosquitoes used for the evaluations of activity had taken at least a partial blood meal, differences in the amount ingested undoubtedly contributed to some variability in the results of different tests. However, repeat tests of the same compound were in general consistent, and greater than two-fold differences in dose-effect relations were unusual (table 7). Fifty per cent or more mosquitoes were killed in 12 out of 22 different tests (55%) with dimethoate at 5 to 6 mg./kg., the ED_{50} level. At double this dosage, 50% or more mosquitoes were killed in 31 out of 34 different tests (91%).

Structure-activity relationships in the carbamoyl alkyl phosphorodithioates tested indicate a marked superiority of O,O-dimethyl compounds in nearly every case over corresponding O,O-diethyl analogs, both regarding degree of safety and margin of safety in the mouse-mosquito test. The few higher alkyls tested were uninteresting. In the O,O-dimethyl series, the methyl substitution of the carbamoyl group was the most active and had the highest margin of safety, excepting the tertiary butyl analog.

Interestingly, the unsubstituted carbamoyl group was less than one twenty-fifth as active as the methyl carbamoyl. Activity decreased somewhat with the ethyl and *n*-propyl as compared with the methyl. The isopropyl, butyl, isobutyl and tertiary butyl analogs were similar to the ethyl in activity. Allyl, phenyl, and cyclohexyl N-substituents were relatively inactive. Other members of the series will be reported subsequently.

It is of interest that two of the metabolites of dimetho-

Table 7.—Reproducibility of activity with dimethoate in the mouse-mosquito test.

Single Oral Dose to Mice (Mg./Kg.)	No. of Tests	Mean No. Mosquitoes Feeding per Test[a]	Per Cent of Tests With	
			100% Activity[b]	50% or Greater Activity[c]
25	18	18±0.9	80	100
10–12	34	17±0.4	41	91
5–6[d]	22	18±0.5	17	55
3	14	17±0.7	7	30

[a] ± standard error.
[b] All mosquitoes dead 72 hrs. after feeding on treated mice 1 hour after dosage.
[c] At least 50% of mosquitoes dead after feeding on treated mice 1 hour after dosage.
[d] The ED_{50}.

ate (personal communication, Professor John E. Casida) *i.e.*, O,O-dimethyl hydrogen phosphorodithioate and O,O-dimethyl hydrogen phosphorothioate, tested as potassium salts were inactive in the mouse-mosquito test at a single oral dose of 250 mg./kg.

Limited data indicate that dimethoate and its O,O-diethyl analog, compound number XIII (see table 3), have approximately equal activity when fed directly to mosquitoes in 5% dextrose solutions. Both killed more than 50% of the mosquitoes at 1 p.p.m. but not at 0.1 p.p.m. The unsubstituted carbamoyl analog in the O,O-diethyl group, compound number XII, killed 50% of the mosquitoes at 0.1 p.p.m. Although differing somewhat from their comparative activities in the mouse-mosquito test (table 3), these results indicate that a direct action of the three compounds *per se* against mosquitoes is possible.

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Soil Insecticides for Plum Curculio Control in Wisconsin[1]

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ABSTRACT

Soil treatments with aldrin and dieldrin in both small plots and in commercial applications gave excellent control of the plum curculio (*Conotrachelus nenuphar* (Hbst.)) for several consecutive years. Heptachlor also appeared promising in these tests. Wettable powder formulations applied in high gallonages of water appeared to give better control than granulated formulations. These insecticides provided more efficient control in cleanly cultivated orchards than in heavily sodded orchards.

Soil treatment to control the plum curculio, *Conotrachelus nenuphar* (Hbst.), was first reported by Snapp (1940), who used dichloroethyl ether. Bobb (1946) was the first to report the use of some of the newer synthetic organic insecticides. He found DDT ineffective at rates up to 25 pounds per acre, but reported 60.9% control with 1 pound per acre of the gamma isomer of BHC. Snapp (1947, 1948) conducted laboratory experiments with BHC, and found it ineffective in preventing the development of the plum curculio in soil. Chandler (1950) found aldrin and dieldrin highly toxic to the plum curculio. Snapp (1953, 1954) reported good control using aldrin, dieldrin, heptachlor and isodrin. Fluke & Dever (1954) showed that aldrin and dieldrin had a residual toxicity of at least 3 years when applied to the soil in controlled cage tests. They reported that aldrin, dieldrin and heptachlor appeared effective in lowering the percentage of curculio infestation in a commercial cherry orchard.

METHODS AND MATERIALS.—The experiments in Wisconsin were begun in 1951 with a series of cage tests at Gays Mills. The methods and materials used for this

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Table 1.—Adult plum curculio emergence from sod treated with insecticides at 6 pounds per acre, Gay Mills, Wisconsin.

Insecticide	Spring Emergence of Adult Curculios				
	1952	1953	1954	1955	Total
Aldrin	0	1	0	1	2
Dieldrin	4	0	0	0	4
Chlordane	285	88	4	9	386
Lindane	67	71	16	71	225
EPN-300	488	139	18	3	648
None	855	63	12	69	999

study have been described in a report of the first 3 years' results (Fluke & Dever 1954). The complete results over a 4-year period are shown in Table 1. In 1952, a similar series of cage tests was started in Door County, Wisconsin. The methods and materials used in this study were described in a report of the first 2 years' results (Fluke & Dever 1954). The results over 4 successive years are shown in table 2.

The above two studies were conducted over heavily sodded soil. Many of the cherry orchards in Door County are kept in clean cultivation and in 1954 an experiment was initiated to determine the control qualities of soil insecticides under this type of cultural practice. A young cherry orchard located on the Peninsular Branch Experiment Station was selected for this study. A ground-spray boom was employed to apply aldrin, dieldrin and heptachlor, each at 2 and 4 pounds per acre, with 500 gallons of water under 400 pounds pressure. The insecticides were applied to four-tree plots during July, 1954. Cages similar to those used in previous tests were placed under the trees, two cages to each plot including the untreated check. Plum curculio infested apples were used to stock the cages. Adult curculio emergence records were taken during August and September. The cages were stocked and similar records taken during the 1955 and 1956 seasons. Table 3 presents the data from these tests.

In the foregoing experiments only wettable powder formulations were used. Similar studies with granulated formulations were conducted in a young apple orchard in Door County. Aldrin, dieldrin and heptachlor at 2.5% 30/60 granulated formulations were applied during October, 1955. The materials were applied with a dry-fertilizer spreader to plots 2/3 acre in size at the rates

Table 2.—Adult plum curculio emergence from insecticide-treated sod, Door County, Wisconsin.

Insecticide	Pounds per Acre	Number of Adults				
		1952	1953	1954	1955	Total
Aldrin	3	0	0	7	4	11
	6	0	0	0	5	5
Dieldrin	3	0	0	0	0	0
	6	0	0	0	7	7
Chlordane	3	6	0	30	137	173
	6	0	0	6	109	115
Lindane	3	9	0	63	97	169
	6	1	1	16	99	117
None	—	147	62	74	127	410

Table 3.—Adult plum curculio emergence from insecticide-treated soil in a cleanly cultivated orchard, Door County, Wisconsin.[a]

Insecticides	Pounds per Acre	Number of Adults			
		1954	1955	1956	Total
Aldrin	2	16	0	0	16
	4	4	0	4	8
Dieldrin	2	31	0	0	31
	4	9	0	0	9
Heptachlor	2	10	0	1	11
	4	5	0	0	5
None	—	165	8	29	202

[a] 400 larvae placed in each cage (800 per treatment) in 1954; 300 larvae placed in each cage in 1955; 280 larvae placed in each cage in 1956.

shown in table 4. This orchard had been completely neglected for 3 or 4 years and had a heavy grass cover at the time the experiment began. During the spring and early summer of 1956 the orchard was disced several times. In July of 1956 four cages were placed in each treated plot and in an untreated check area. These cages were stocked with plum curculio infested fruits. Adult emergence records obtained in August and September of 1956 are given in table 4.

Discussion.—The results at Gays Mills (table 1) show that aldrin and dieldrin gave nearly perfect control through the fourth year after treatment. Lindane gave about 92% control the first year but thereafter was no better than the untreated check. Chlordane and EPN-300 did not give promising results in this study. The Door County cages (table 2) also showed aldrin and dieldrin superior to chlordane and lindane. Chlordane, however, gave excellent control at both 3 and 6 pounds in 1952 and 1953; in 1954 its control qualities were limited, and in 1955 it was no better than the check. The results from the use of lindane paralleled those obtained with chlordane in this test. Chlordane and lindane both appeared to have a longer residual toxicity in Door County soil than in Gays Mills. Fluke & Dever (1954) pointed out the possibility that the difference in soil types between Gays Mills and Door County may account for the differences noted in the toxicity of chlordane and lindane.

The emergence records from cages placed over treated soil in a cleanly cultivated orchard (table 3) indicated that low doses of insecticides may provide excellent control of the plum curculio under such conditions. In 1954, the year the treatments were applied, all treatments were significantly better than the check (1% probability level).

Table 4.—Adult plum curculio emergence from sod treated with granulated insecticides, Door County, Wisconsin.

Insecticide	Pounds per Acre	Replicates				
		I	II	III	IV	Total
Heptachlor	5.0	0	2	8	0	10
Dieldrin	5.6	6	7	3	0	16
Aldrin	5.6	6	13	11	14	44
None	—	20	11	12	15	58

Aldrin and heptachlor at the rate of 4 pounds were significantly better than the other treatments. During the next 2 years the emergence was poor from all the cages including the untreated check. It is possible that the check plot became contaminated through the cultivating process since there were only two rows of trees separating the treated areas from the check area. There is also the possibility that insecticide-treated soil from different plots became mixed. It may be concluded, however, that 2 to 4 pounds per acre of aldrin, dieldrin or heptachlor will provide excellent control of the plum curculio for several years in cleanly cultivated orchards.

Dieldrin and heptachlor applied in granulated fromulations to the soil of a heavily sodded orchard were also effective in controlling the plum curculio (table 4). However, the degree of control was less than that expected from similar doses of these chemicals applied as wettable powders. Aldrin was ineffective in this test.

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Time of Insecticide Applications in European Corn Borer Control[1]

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ABSTRACT

Experiments to study time of insecticide application in relation to control of the European corn borer (*Pyrausta nubilalis* (Hbn.)) were carried out at Ankeny, Iowa, in 1951–56.

Two applications of DDT gave significantly better borer control than one application in all experiments. Each year the best control was obtained following applications when egg laying was almost completed.

The minimum points on regression curves represented the estimated optimum treatment dates. Criteria used for predicting time of insecticide application, as they existed on the dates of optimum treatment, were compared with the recommended or suggested criteria.

In 5 out of 6 years the number of borer-degree days on the date of optimum treatment was below the suggested 1100, averaging about 900.

A tassel bud-plant height ratio of 35 appeared to be more suitable than the recommended range of 30 to 50. Except for 1 year plants in plots with the best borer control were consistently taller than the recommended 35 inches.

Plant height and unhatched egg masses were found to be unreliable.

Data on moth emergence were consistent with recommendations; however, this criterion is unsuitable for use by the average grower.

The recommendation to treat when 75% of the plants show evidence of recent feeding in the whorls was the most rapid and simple to use.

Various investigators have attempted to find a foolproof means of determining the proper time to apply insecticides for control of the European corn borer (*Pyrausta nubilalis* (Hbn.)). The following criteria have been suggested or recommended by different workers: (1) 1100 borer-degree days, (2) tassel-bud ratio of 30 to 50, (3) corn plant extended height of 35 inches, (4) 50 to 100 accumulated egg masses per 100 plants, (5) 87% moth emergence, (6) 75% of plants with leaf-feeding damage, and (7) first egg hatching. Each criterion has been used with some success, but also has had its limitations.

Entomologists in different States in the Corn Belt have placed emphasis on one or another of these criteria, and the apparent disagreement between specialists has confused corn growers. Further, climatic factors have sometimes entered the picture to alter the results of recommended procedures based on certain of the criteria. In addition, many corn growers are either unable or unwilling to use such recommended practices as counting egg masses, calculating borer-degree days, or determining tassel-bud ratios. These practices have generally been more acceptable to sweet corn canners or seed corn growers than to growers of field corn or market sweet corn.

Studies on the timing of insecticide applications were carried out from 1951 through 1956 at the European Corn Borer Research Laboratory, Ankeny, Iowa. The recommended procedures were compared to determine if one was better than the others.

REVIEW OF LITERATURE.—Most current recommendations for applying insecticides to control the European corn borer are based on the number of egg masses per

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FIG. 1.—Nozzle arrangement used for all experiments. Spray patterns crossed about 6 inches above the plant whorl.

100 plants. However, increasing emphasis is being placed on the number of plants with leaf-feeding damage.

The North Central Technical Committee on Entomology (1955) and the Entomology Research Branch (1955) recommend a single treatment 10 to 14 days after the first egg hatch, provided there are 50 or more egg masses per 100 plants present, or when 75% of the plants show evidence of recent feeding in the whorl. Spraying 10 to 12 days after first egg hatching in fields having 50 egg masses per 100 plants was recommended by Holdaway *et al.* (1949). Bigger *et al.* (1947) obtained the best control when insecticides were applied 10 to 15 days after the finding of approximately 100 egg masses per 100 plants. Decker & Bigger (1949) recommended treating 10 to 14 days after the first egg hatch in fields with plants at least 25 inches tall and which had an average of 100 egg masses per 100 plants, Chiang & Hodson (1952) believed the critical level of 50 egg masses per 100 plants should be reviewed with consideration of such factors as cost of treatment, efficiency of expected control, relationship between egg-mass counts and yield loss, normal land yield value of corn crop, and complication of infestation by second-brood borers. Chiang & Cutkomp (1953) concluded that under all weather conditions consistently good borer control would not be possible by applying an insecticide a fixed number of days after the first hatching.

Although Ditman & Lloyd (1951) found no apparent relation between time of heaviest egg deposition and the effectiveness of a single spray application, they did note a relation between development of corn plants and the best time of treatment. Painter & Wilbur (1950) reported that corn would be between 2 and 3 feet in extended height when the deposition rate was 50 egg masses per 100 plants. The North Central Technical Committee on Entomology (1955) reported that only in very severe infestations would treatment of plants less than 35 inches be justified.

Hawkins (1951) obtained the best control with a single application when 13 to 52% of the eggs had hatched. On the other hand, Apple (1953) regarded 75% egg hatching as the critical level for maximum control.

Hawkins & Thurston (1949) suggested the use of cumulative percentage frequency curves for moth emergence in relation to oviposition and hatching as aids in predicting the time of insecticide applications. Hawkins (1951) obtained the best control by applying insecticides when 87 to 99% of the moths had emerged.

Chiang & Hodson (1953) studied and suggested the use of leaf injury as an index to the best time for insecticide applications to field corn. The North Central Technical Committee on Entomology (1955) and the Entomology Research Branch (1955) recommendations on leaf damage were cited above.

Luckmann & Petty (1957) recommended treating field corn when the ratio of plant height to tassel-bud height is between 30 and 50 if 75% or more of the plants show recent feeding in the whorl leaves. They suggested that best control would be obtained when the ratio was between 40 and 50.

Apple (1953) proposed the use of temperature accumulation (borer-degree days) as a means of timing application. He found the best control was obtained when applications were made at approximately 1100 borer-degree days.

MATERIALS AND EQUIPMENT.—A 25% DDT emulsifiable concentrate was used in all experiments. The rate of application for all treatments was 1½ pounds of DDT in 10 gallons of water per acre. The material was applied under a pressure of 40 pounds per square inch.

Applications were made with a high-clearance sprayer modified to treat four rows of corn simultaneously. Two 65° fan-type nozzles were mounted on either 3- or 4-inch drops over each row. The nozzles were calibrated each year to deliver the desired amount of spray. Nozzle arrangement and spray pattern are shown in figure 1.

METHODS.—A randomized complete-block design was used each year except 1956, when a split-plot design was used. In all years each treatment was replicated four times. The plots were four rows wide by 140 feet long. Although granules and emulsions were tested in both 1955 and 1956, only the results with emulsions are reported herein. The corn variety was Ia-4297.

Ten plants were tagged at random in each untreated plot. The plants were examined each Monday, Wednesday, and Friday, and the numbers of new, hatched, and missing egg masses were recorded. Indelible ink was used to mark the location of the egg masses on the leaves. On alternate days six to eight plants in each untreated plot were measured and dissected to determine extended height, natural height, and height of the tassel bud.

As each plant was checked for egg masses, the whorl was observed for evidence of feeding (shot holes) by newly hatched borers. A record was made of the date when shot holes were first found on each plant. It was possible to determine the percentage of the plants showing feeding damage by a day-to-day summation of plants with new damage out of the 10 plants being examined in each plot.

Borer-degree days are simply accumulation of temperatures taken from the daily weather records during the period of borer development. The daily maxima and minima were averaged and the degrees above 50° F. added day by day.

Moth emergence was determined periodically by dissecting corn stubble in oat fields and counting the empty pupal cases out of 100 pupal forms.

When most of the larvae were full grown, 10 plants were taken at random from each plot in 1951, 1952, and

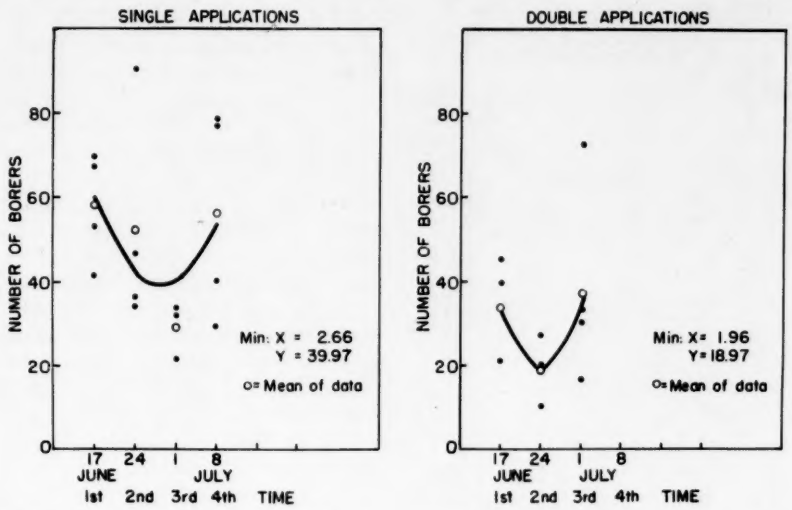

FIG. 2.—Example of calculated regression curves plotted for borer-control data in each experiment. Lowest point on each curve represents optimum treatment date.

1953, and 20 plants in 1954, 1955, and 1956. The plants were dissected and the numbers of larvae and tunnels in each plant were recorded. Since there was a high correlation between the number of larvae and the number of tunnels, only the data on larvae are reported herein.

An analysis of variance was performed on the data for each year. An F-test was made to determine if differences in control existed between different dates of application. In addition, the linear and quadratic components for both single and double applications were tested. After determination of the type of regression best fitted to the data available, the data were plotted and a curve was drawn through the points representing the calculated regression. From the minimum point on each curve it was possible to estimate the date of application on which maximum borer control would have been obtained. Figure 2 is an example of the plotted data.

RESULTS.—The most commonly suggested or recommended timing criteria—extended plant height, tassel-bud ratios, and accumulated egg masses—as they existed on the dates when the best corn borer control was obtained each year are shown in the accompanying tables.

1951 (table 1A).—All the treatments except the single application on June 12 and the double applications on June 12 and 19 significantly reduced the borer population below that in the untreated plots. Although the smallest number of borers following a single application were found in the plots treated on June 26, there was no significant difference between these plots and those treated on July 5 or 10. Furthermore, although the plots treated on both July 10 and 17 were devoid of borers, there was no difference between these plots and those treated on July 5 and 10 or June 26 and July 5. The double applications gave significantly better control than the single applications.

The best single treatment was applied to corn 44 inches in extended height with a tassel-bud ratio of 15.0 and an accumulated egg deposit of 140 masses per 100 plants. These criteria differed considerably from those recommended or suggested in the literature.

1952 (table 1B).—Treatments on all dates significantly reduced the borer population below that in the untreated plots. The best control from a single application was obtained on June 19, and from double applications on June 19 and 28. The double applications gave significantly better control than the single applications.

Although the best control was actually obtained on June 19, the optimum treatment date was June 12, as estimated from a linear regression giving the best fit for the available data. However, had control data been taken on several dates earlier than June 12, a quadratic regression would probably have resulted since the other experiments indicate that early applications result in poor control. With a quadratic regression the minimum point would then have been about June 19. On that day the extended plant height and accumulated egg deposit were extremely close to the recommended figures. The tassel-bud ratio was far below the recommended range.

1953 (table 1C).—All the treated plots had significantly lower borer populations than the untreated plots. The plots receiving single applications on June 19 and 26 contained the fewest borers. The best control from a double application was also obtained from treatments applied on these dates. However, it was only slightly better than the bouble applications on June 12 and 19 and on June 26 and July 3. Double applications gave significantly better control than single applications.

At the times the best single treatments were applied the plants were taller, had lower tassel-bud ratios, and had more egg masses per 100 plants than corresponding recommended criteria.

1954 (table 1D).—The treated plots had significantly lower borer populations than the untreated plots. There were also significant differences between single applications on different dates, the best control being in plots treated on July 1. Although the differences between dates of application of double treatments were not significant, fewer borers were found in plots treated on June 24 and July 1. Double applications gave significantly better control than single applications.

Table 1.—Control of first-brood European corn borer larvae by spraying with DDT emulsion on different dates in 1951–56, inclusive, and status of three criteria used to determine the time of application.

Application Dates	Number of Larvae per 100 Plants	Per Cent Control	Extended Plant Height (Inches)	Tassel-Bud Ratio	Accumulated Number of Egg Masses per 100 Plants
		A. 1951			
June 12	92	0	21	—	10
19	37	40	35	4.2	54
26	5	92	44	15.0	140
July 5	10	84	59	40.9	168
10	7	89	69	62.6	169
17	22	65	—	—	169
June 12 and 19	55	11			
19 and 26	5	92			
26 and July 5	2	97			
July 5 and 10	2	97			
10 and 17	0	100			
Untreated plots	62	—			
		B. 1952			
June 12	37	59	31	3.5	33
19	2	98	37	9.2	47
28	42	53	59	42.7	50
July 3	47	48	57	43.2	50
10	62	31	80	85.3	50
June 12 and 19	12	87			
19 and 28	0	100			
28 and July 3	10	89			
July 3 and 10	37	59			
Untreated plots	90	—			
		C. 1953			
June 12	52	53	29	2.5	15
19	15	86	45	14.1	112
26	15	86	55	34.5	157
July 3	32	71	82	50.2	162
10	50	55	88	83.9	162
June 12 and 19	5	95			
19 and 26	0	100			
26 and July 3	2	98			
July 3 and 10	15	86			
Untreated plots	110	—			
		D. 1954			
June 17	287	25	29	2.8	55
24	230	54	43	6.1	291
July 1	145	62	59	25.9	350
8	280	27	76	58.7	353
June 17 and 24	170	56			
24 and July 1	95	75			
July 1 and 8	184	52			
Untreated plots	384	—			
		E. 1955			
June 2	334	19	26	1.6	49
9	164	60	35	4.7	276
14	141	66	39	11.3	330
19	71	83	48	23.9	410
24	55	87	56	37.1	475
June 2 and 9	192	54			
9 and 14	139	66			
14 and 19	27	93			
19 and 24	40	90			
24 and July 1	21	95			
Untreated plots	414	—			
		F. 1956			
June 9	109	0	—	—	8
11	81	9	—	—	20
13	76	15	—	—	40
15	107	0	40	6.1	48
17	40	55	—	—	52
19	34	62	—	—	56
21	24	73	50	30.8	59
23	14	84	—	—	60
25	10	89	62	34.7	60
27	45	49	—	—	60
Untreated plots	89	—			

When the best control was obtained, on July 1, the plants were taller than the recommended 35 inches and had considerably more egg masses per 100 plants, and the tassel-bud ratio was still below the recommended figures.

1955 (table 1E).—The treated plots had significantly lower borer populations than the untreated plots. In plots receiving a single application the fewest borers were found in the plants treated on June 24. Although the plants treated with a double application on June 24 and July 1 had the smallest number of borers, the control was not significantly better (at 19:1) than from double applications on June 14 and 19, and on June 19 and 24.

There were significant differences in control obtained on the different dates for both single and double applications. Single applications on June 19 or later and double applications on June 14 and 19 or later gave better borer control than those made earlier. The double applications gave significantly better control than the single applications.

On June 24 the plants had an extended plant height and an accumulated egg deposit far in excess of the recommendations, but in spite of these differences the tassel-bud ratio was in the range of 30 to 50.

1956 (table 1F).—There were significant differences in the control obtained on the different dates of application. Control was either very poor or nonexistent in plots treated on June 9, 11, 13 and 15. Although the fewest borers were found in plots treated on June 25, there was relatively little difference in the control obtained on June 25 and June 23.

The accumulated egg deposit on June 25 was within the recommendation. However, had only a single count been taken (a method utilized by some farmers) at any time during the egg-laying period, there would never have been 50 eggs per 100 plants. Since borer eggs hatch in 5 to 7 days, the rate of hatching would have been too great to permit a buildup to 50 masses.

The extended plant height on June 25 was 27 inches more than 35 inches. The tassel-bud ratio was within the suggested 30 to 50.

Comparison of Timing Criteria.—The optimum treatment dates for the 6 years are summarized in table 2. For two applications the calculated optimum date is the date of the first treatment. For example, in 1951 since the interval between treatments was 7 days, optimum control would have been obtained by treating on June 3 and 10.

The recommended figures for criteria used to predict time of application and the values actually existing on optimum treatment dates are also summarized in table 2. When no data had been obtained on the calculated optimum treatment date, appropriate estimates were made.

The number of borer-degree days on the best date of treatment was below the suggested figure of 1100 in 1951 through 1955. Only in 1956 did the figure approximate 1100. Therefore, based on means of borer-degree days on the optimum treatment dates, a figure of 890 to 910 would appear to be more realistic.

The tassel-bud ratio was below the recommended 30 to 50 on the calculated optimum treatment dates of June 12 and 20, 1952, June 20, 1953, and June 24, 1954. Although the ratio varied greatly from year to year, the one occurring most frequently was close to 35; the average for all years was 28. On the basis of these results, a recommendation to treat when the tassel-bud ratio reached 35 would appear to have more merit than the range recommended.

With the exception of the June 12 treatment date in 1952, plants in plots with the best borer control were consistently taller than 35 inches. Variations in planting dates and the use of hybrids adapted to different growing seasons almost preclude the use of plant height as a criterion for estimating treatment dates. However, if it is to be used, it should probably be applied only to early-planted corn and a figure between 45 and 50 inches would appear to be more realistic.

Table 2.—A comparison of recommended figures for criteria used to predict time of insecticide application for maximum control of first-brood European corn borer and the values actually existing on the optimum treatment dates.

Year and Number of Applications	Calculated Date on Which Optimum Control Was Obtained	Borer-Degree Days	Tassel-Bud Ratio	Extended Plant Height (Inches)	Accumulated Number of Egg Masses per 100 Plants	Moth Emergence Per Cent of Total Borer Forms	Per Cent of Plants with Feeding Damage on Leaves
1951							
1	July 6	1001	45.3	61	168	92	—
2	3	956	35.3	55	161	90	—
1952							
1	June 12	722	3.5	31	33	60	—
2	20	905	12.6	39	47	80	—
1953							
1	June 26	945	34.5	55	157	93	—
2	20	822	17.6	46	118	76	—
1954							
1	June 29	1048	35.5	54	342	93	84
2	24	892	14.0	43	291	58	36
1955							
1	June 24	875	37.1	56	480	—	100
2	24	875	37.1	56	480	—	100
1956							
1	June 25	1087	34.7	62	60	—	38
Suggested or recommended	—	1100	30–50	35	50–100	87–99	75

Egg deposition varies considerably from year to year. Under certain climatic conditions eggs are deposited over a protracted period. As a consequence any one observation in a field may never reveal 100 or even 50 egg masses per 100 plants, even though the total deposit may be two or three times these figures. Therefore, a farmer might never find sufficient eggs to justify treatment if he used only the criterion of a certain number of egg masses. Its use appears to be justified only in years when eggs are being deposited at a high rate or when egg counts are made on a cumulative basis to determine both the total number deposited and when deposition is practically completed.

Although moth emergence was recorded for only 4 years, the percentages appear to be consistent with the suggested criteria. However, this method is obviously unsuitable for grower usage. The observations must be made by an entomologist or specially trained personnel. A further limitation is that the results are applicable only to localized areas.

Counts of plants showing evidence of leaf feeding by newly hatched larvae were made in only 3 years. The percentages varied considerably from the recommended 75%. However, in other experiments during the same 3-year period very good results were obtained by treating when 75% or the plants showed leaf damage. As a single method of determining when to treat, this criterion has the advantage of being most simple and rapid of all the methods compared. Although not shown in these experiments, it is at least as effective as any of the other criteria.

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Further Studies with Systemic Insecticides against the Green Peach Aphid on Potatoes[1]

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Abstract

Tests for the control of the green peach aphid (*Myzus persicae* (Sulz.)) on late-crop plantings of potatoes were made at Prosser, Wash. Satisfactory control was either not obtained or, if so, only for 3 to 7 days following applications of several dosages of schradan, demeton, Thimet, Guthion, Am. Cyanamid 12008, Chlorthion, and parathion in 1955, and Diazinon, schradan, and combinations of schradan and Diazinon in 1956 during the period that the numbers of winged migrants present in the area steadily increased. After the peak of aphid migration had been passed and the numbers of winged aphids steadily diminished to the end of the season, many treatments that had previously been unsatisfactory gave good control for 13 to 17 days.

Over a period of years the green peach aphid (*Myzus persicae* Sulz.) has become increasingly hard to control on potatoes and several other crops in central Washington. There are several plausible explanations for this, but none seems entirely satisfactory.

In 1949 Gibson *et al.* (1951) obtained good control of this aphid with a DDT-sulfur dust. In later tests Klostermeyer (1953) found DDT, DDT-sulfur, and DDT-sulfur-parathion dusts fairly satisfactory, but not nearly so effective as schradan or demeton sprays. Then Anthon (1955) reported that, although malathion, parathion, and TEPP sprays had given good control of this aphid on peach trees near Wenatchee, Wash., in 1953 the results suddenly became poor or erratic. In preliminary tests Anthon obtained good results with nicotine or schradan sprays. In 1954 Klostermeyer *et al.* (1956) compared demeton, schradan, Isolan, and parathion at three dosage levels, but none of these treatments gave satisfactory control after repeated applications. Schradan was the most promising insecticide tested in 1954, but both it and demeton were far less effective than in 1951 (Klostermeyer 1953), and neither was superior to parathion after the first week.

This paper is a report of further tests with schradan and other systemics, in comparison with parathion, conducted at the Irrigation Experiment Station, Prosser, Wash., in 1955 and 1956.

General Methods.—The experiments were conducted on the Russet Burbank variety of potatoes planted the last week of May for late crops. Seed pieces were planted in rows 32 inches apart and spaced 9 inches in the rows. Treatments were started when the plants were 4 inches high. Emulsions were applied with a Slosser-type sprayer. Four nozzles were used on each row. Since there was some overlapping of treatments on the two outside rows, they were never used for sampling. Results were checked by making two counts 3 or 4 days apart of the winged and wingless forms found on 50 compound leaves per plot after each application. A leaf was picked at random from the lower half of each of 25 random plants along the third row and 25 from the fifth row of each plot.

1955 Experiment.—In 1955 plots 7 rows wide and 63 feet long were arranged in randomized blocks and treatments were replicated five times. Demeton and schradan received only the first two of three applications. The plots were sprayed at 25 gallons per acre on July 12 and 22 and August 5. Twelve counts were made from June 30 to September 1.

Counts made after the first application as shown in table 1, indicated that the dosages might be too low. Therefore, dosages for the second application were doubled for all insecticides except parathion, demeton, and schradan, which had shown some promise at the rates used. For the third application the use of parathion at the original 3/4-pound rate was continued for comparative purposes, but dosages of Thimet, Am. Cyanamid 12008, and Chlorthion were increased to 1½ pounds per acre. Since two applications of demeton and schradan had given fairly good control of the aphids, the third application was omitted to determine the duration of the residual action of these systemic insecticides.

Good control was obtained with the third application of Thimet, Am. Cyanamid 12008, and Chlorthion, at which time very few winged aphids were present. This might indicate that the previous dosages of these materials were too low, except that parathion also gave exceptionally good control at the 3/4-pound rate at which it had previously given poor control. The better performance of the insecticides in this application seemed to be due to the low influx of winged migrants.

Tremendous numbers of winged migrants were produced on an early planting of potatoes ½ mile to the west, or windward, side of the test plots. Migration of aphids from this field started about the time the first application of insecticides was made on the test plots. Although few winged aphids are collected in leaf samples, their numbers usually vary in proportion to the intensity of flight at the sampling location. In these tests the populations showed a rapid increase, reaching a peak on July 29, or just a few days before the third application. During the entire period practically all the wingless aphids found on the leaves picked 3 days after treatment were small, and presumably newly deposited, whereas after 6 or more days both large and small aphids were taken. Many aphids were killed at the time of treatment, and they invariably fell off the plant within a short time after death. Many of the aphids that were deposited on the potato foliage after treatment were not killed immediately, or even after a day or so of feeding, regardless of what insecticide had been applied. Therefore, few dead aphids were ever observed in the samples, although 24 hours may have elapsed between the collection and the counts.

The constant replenishment of the populations of wingless aphids by tremendous numbers of winged aphids

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Table 1.—Control of the green peach aphid on Russet Burbank potatoes with insecticides, 1955.

Material	First Application (July 12)			Second Application (July 22)			Third Application (August 5)		
	Pounds per Acre	Number of Surviving Aphids per 250 Leaves		Pounds per Acre	Number of Surviving Aphids Per 250 Leaves		Pounds per Acre	Number of Surviving Aphids per 250 Leaves	
		July 15	July 19		July 25	July 29		Aug. 8	Aug. 12
Schradan	2	80	2,031	2	443	1,143	0	490	1,282
Demeton	1	37	1,982	1	576	662	0	5,234	4,220
Thimet	0.5	441	8,852	1	5,674	16,630	1.5	52	1,286
Guthion	0.5	758	6,082	1	13,487	13,472	1.5	8,886	11,194
Am. Cyanamid 12008	0.5	858	7,373	1	3,492	8,829	1.5	26	716
Chlorthion	0.5	534	6,873	1	18,313	19,013	1.5	22,958	20,960
Parathion	0.75	261	7,504	0.75	8,295	25,118	0.75	654	3,324
Check (untreated)	0	1,927	7,355	0	44,046	62,170	0	36,583	5,618
L.S.D. at 5% level		681	2,566		14,207	16,222		22,910	10,148

during the period of heavy migration and the slow rate of kill of the aphids deposited after the plants had been treated were believed to have been largely responsible for differences in control obtained from one part of the season to another.

Schradan appeared to be the best of the systemic insecticides in the 1955 tests.

1956 EXPERIMENT.—Previous unreported tests had shown that Diazinin, when used at twice the strength of parathion, was more effective against the green peach aphid than parathion. In an effort to obtain quick kill of the aphids and a long residual action against reinfestation, Diazinon was mixed with schradan in various proportions and applied in sprays at the rate of 20 gallons per acre. There were 16 treatments replicated in three randomized blocks. The plots were 7 rows wide and 45 feet long and were sprayed on June 29, July 13, and August 3. The first application was made 2 weeks earlier and the intervals between applications were longer than in 1955.

The principal source of winged aphids was a commercial potato field ½ mile west of the plots. Field observations showed that the migration from this field was well under way when the plots were treated the first time, and reached a peak on July 12, somewhat earlier than in 1955. In the untreated plots the peak population of wingless aphids occurred about July 18, or only a few days after the peak of migration. Table 2 shows that all the treatments were less effective while winged aphids were migrating into the plots before the second application than after migration abated.

Schradan was found to be somewhat more effective than Diazinon, but neither of these materials when used alone or in combination gave satisfactory control of the aphids during the period of heavy migration. More than ½ pound of Diazinon per acre was required to give good initial kill and even the 1- and 2-pound dosages failed to deter reinfestation for more than 3 to 6 days. After the residual effect of the Diazinon wore off, the aphids increased rapidly. Fourteen days after the second application the counts of aphids in the Diazinon-treated plots were higher than the pretreatment counts and the heaviest population developed where the most Diazinon had been applied. Schradan also was unsatisfactory at the ½-

Table 2.—Number of surviving green peach aphids per 250 potato leaves following three spray applications with Diazinon and schradan, alone and in combination, 1956.

Materials and Pounds per Acre		Days After June 29			Days After July 13			Days After August 3		
Schradan	Diazinon	3	7	13	3	7	13	3	7	17
0	0	2,199	4,995	11,725	13,777	2,927	3,935	29	10	58
	0.5	418	3,176	11,489	1,671	4,433	806	71	106	67
	1	68	1,734	12,004	417	2,219	2,596	16	42	172
	2	26	1,271	10,883	151	1,408	3,267	0	4	101
0.5	0	371	794	6,686	365	495	1,136	7	8	46
	0.5	224	609	5,538	31	212	991	1	3	18
	1	17	309	8,911	15	495	1,091	0	5	118
	2	5	26	5,067	9	152	789	0	1	10
1	0	63	194	7,166	34	146	391	0	0	31
	0.5	11	47	3,255	97	237	221	0	1	2
	1	6	9	3,047	1	25	110	0	0	4
	2	3	15	3,848	0	72	343	0	1	16
2	0	36	71	3,969	7	92	139	0	0	4
	0.5	0	32	4,787	1	63	147	0	0	16
	1	2	16	1,794	9	8	75	0	0	0
	2	0	7	3,078	4	45	19	0	1	4
L.S.D. at 5% level		451	2,330	4,715	1,157	1,242	—	—	—	—

pound dosage; 1 and 2 pounds of schradan gave fairly good control for about 6 days. Mixtures of schradan and Diazinon gave no better control than when used separately.

As in 1955, after the peak flight had passed the effectiveness of the insecticides appeared to persist for a much longer time. Even so, schradan and Diazinon applied separately at ½-pound rates gave relatively poor initial kill, whereas schradan alone at 1 pound or in combination with varying dosages of Diazinon gave nearly 100% control for 3 to 7 days. There was no indication of antagonism between Diazinon and schradan, nor did the combination of the two insecticides offer any practical advantage.

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The Influence of Yeast Hydrolysate on the Behavior of the Melon Fly[1]

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Abstract

This study, which was conducted to determine some effects of yeast hydrolysate on the behavior of the melon fly (*Dacus cucurbitae* Coq.) in the field, showed that the number of flies present or found dead was greater when yeast hydrolysate bait was applied to non-host plants favored by the adults than to favored host plants or to plyboard slabs. The application of yeast hydrolysate to non-host plants, fuzzy rattle pod (*Crotolaria incana* L.) and corn, did not result in an increase of flies on the treated plants in certain tests, but in others there was an increase. In general, positive responses were found to be associated with high population density. The application of this bait to host plants, cucumber and cantaloupe, did not result in the movement of flies to these crop plants from nearby untreated corn plants.

Yeast hydrolysate, a material which contains principally a mixture of various amino acids and vitamins, was first investigated in Hawaii by Hagen & Finney (1950) who used it in investigations on the nutrition of fruit flies. They found that fruit flies, including the melon fly, *Dacus cucurbitae* Coq., fed on this material quite readily, and that when included in the diet it caused a marked increase in fecundity. Subsequently, this material was investigated as a bait spray for the control of fruit flies (Steiner 1952, 1954, 1955, Nishida *et al.* 1957). These later studies were concerned primarily with an evaluation of the effectiveness of yeast hydrolysate when used as a bait for the control of the melon fly as well as other fruit flies. The present paper presents results of studies conducted on the behavior of adult melon flies as influenced by field applications of yeast hydrolysate.

General Procedure.—Studies were conducted on private farms as well as on the University of Hawaii farm at Waimanalo, Oahu, during 1955 and 1956. All experiments were conducted during May to September when there was little or no rain to wash off the residue or to affect fly activity adversely. Sprays containing yeast hydrolysate alone and in combination with 25% malathion wettable powder were used. In experiments where individual plants were treated, approximately 350 cc. of spray was applied per plant, using a knapsack sprayer; in those experiments where plots were treated, sufficient spray was applied to wet the upper and lower surfaces of the leaves, using a 25-gallon capacity power sprayer. In order to reduce drift as much as possible the applications were made at low pressures and by use of a nozzle with a large aperture. In experiments where individual plant treatments were made, the plants were shaken prior to treatment in order to drive away the flies on them. The check plants were likewise shaken, but no spray was applied to them.

Response of Flies to Yeast Hydrolysate.—The response of melon flies to yeast hydrolysate was studied in an abandoned watermelon field where melon flies were present in large numbers on various weeds. Individual fuzzy rattle-pod plants (*Crotolaria incana* L.) were sprayed in three replications with yeast hydrolysate at concentrations of 1, 2, and 4 pounds per 100 gallons of water. The mean number of flies counted on the treated and untreated plants at various intervals of time is presented in figure 1.

The results obtained show that the number of flies counted on plants sprayed with yeast hydrolysate was greater than that on the untreated. However, this condition was temporary, for the number of flies on the treated plants decreased rapidly with time. The presence of melon

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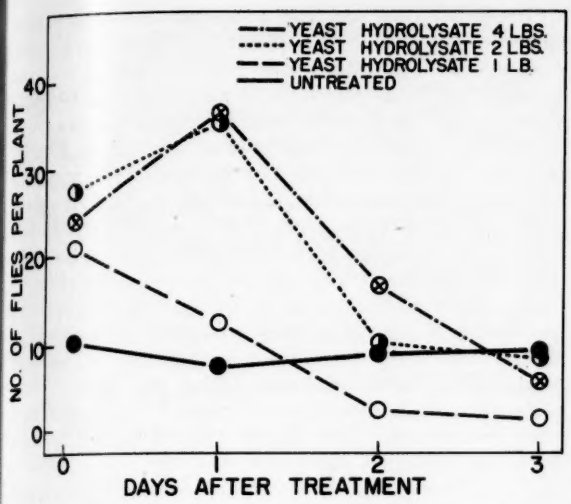

FIG. 1.—Melon fly response to various concentrations of yeast hydrolysate when approximately 350 cc. of spray per plant was applied to fuzzy rattle pod (*Crotolaria incana* L.).

flies on the check plants and on other untreated plants adjacent to those that were sprayed indicated that not all individuals in the population responded to the bait.

EFFECT OF THE SUBSTRATE ON RESPONSE.—Two studies were conducted on the effect of the substrate on the response of the melon fly to yeast hydrolysate. One was to determine the effect of different plant species and the other to determine whether or not there was a difference in response between plants and wooden slabs.

The first study was conducted by spraying individual plants of different species with a mixture containing 2 pounds of a 25% malathion wettable powder and 1 pound yeast hydrolysate per 100 gallons and then counting the number of flies that died upon feeding on the sprayed residue at various intervals of time. The data obtained by spraying 10 plant species commonly found in the Waimanalo area, presented in table 1, indicate that the greatest response was obtained when the bait was applied to non-host plants such as *Euphorbia*, pigeon pea, cocklebur, corn, and fuzzy rattle pod, all of which are species recorded as highly favored by adult melon flies (Nishida & Bess 1951, Nishida 1954, Nishida & Bess, in press). Surprisingly, the response was poor when the bait was applied to watermelon and cucumber even though these are favorable host plants of the melon fly.

Table 1.—The number of dead melon flies counted under various plants sprayed.[a]

PLANTS SPRAYED	TOTAL FLIES COUNTED	MEAN NO. OF DEAD FLIES PER PLANT AFTER (HR.) 1	3	5
Watermelon field				
Watermelon (*Citrulus vulgaris* Schrad.)	45	3.3	4.0	7.7
Wild Euphorbia (*Euphorbia geniculata* Ortega)	739	101.0	49.0	96.3
Spiny Amaranth (*Amaranthus spinosus* L.)	187	18.0	19.0	25.3
Popolo (*Solanum nodiflorum* Jacq.)	183	12.0	16.0	33.0
Cucumber field				
Cucumber (*Cucumis sativus* L.)	34	3.3	4.3	3.7
Fuzzy Rattle Pod (*Crotolaria incana* L.)	218	8.0	31.7	33.0
Corn (*Zea mays* L.)	265	10.7	32.7	45.0
Pigeon Pea (*Cajanus indicus* Spreng.)	516	65.0	55.0	52.0
Napier grass (*Pennisetum purpureum* Sch.)	52	11.0	3.3	3.0
Cocklebur (*Xanthium saccharatum* Wall.)	816	139.0	75.7	57.3

[a] Three replications with approximately 350 cc. of spray containing 2 pounds of a 25% malathion powder and 1 pound yeast hydrolysate per 100 gallons.

Table 2.—The number of dead flies counted under various plants and plywood slabs treated with a malathion-yeast hydrolysate spray.

TEST NO.	BAIT SPRAY APPLIED TO:	NO. OF DEAD FLIES PER THREE REPLICATIONS AFTER 1 HR.	2 HRS.
1	Plywood slabs in open field	0	0
	Plywood slabs among corn plants	0	0
	Corn plants (*Zea mays*, L.)	17	4
2	Plywood slabs in open field	0	0
	Plywood slabs among cocklebur	0	0
	Cocklebur (*Xanthium saccharatum* Wall.)	3	4
3	Plywood slabs in open	0	0
	Plywood slabs among spiny amaranth	1	2
	Spiny amaranth (*Amaranthus spinosus* L.)	3	7

The second study, designed to compare the response when yeast hydrolysate was applied to certain plants and plyboard slabs, was conducted in a recently plowed tomato field surrounded by volunteer corn, cocklebur, and spiny amaranth plants. Plyboard slabs (6×8 inches), treated with malathion-yeast hydrolysate spray, were suspended approximately 5 feet from the corn, cocklebur, and spiny amaranth plants, and also a foot away from these plants in three replications. An equal number of corn, cocklebur, and spiny amaranth plants were also sprayed with the malathion-yeast hydrolysate mixture and, as in the previous study, the numbers of dead flies on the ground were counted. The results of this study, presented in table 2, show that the flies did not respond to the residue on plywood slabs placed either in open sites or among corn and cocklebur plants, except in test 3 in which few flies were counted. Evidently, the flies responded to these slabs under conditions of high population density, for it may be seen from table 2 that the total number of flies counted was highest in test 3, the only one in which a positive response was obtained.

EFFECT OF YEAST HYDROLYSATE ON FLY MOVEMENTS.—Studies on the effect of yeast hydrolysate on melon fly movements were conducted to determine the extent, if any, to which this bait induces movements from (1) untreated non-host plants to treated non-host plants, (2) untreated non-host plants to treated host plants, and (3) the untreated half of a field to the treated half.

Movements among non-host plants.—This study on the movement of flies from untreated corn to yeast hydrolysate-sprayed corn plants was conducted in a cantaloupe field in which two rows of corn approximately 3 feet apart were planted along the periphery. Eight 60-foot corn plots were staked out along each of the northeast and southwest sections of the field. In each section four plots were sprayed with yeast hydrolysate at the concentration of 1 pound per 100 gallons and the remaining four left untreated. Prior to treatment daily records were taken on the number of adults on the corn plants for 8 days and on the ninth day yeast hydrolysate was applied to all ex-

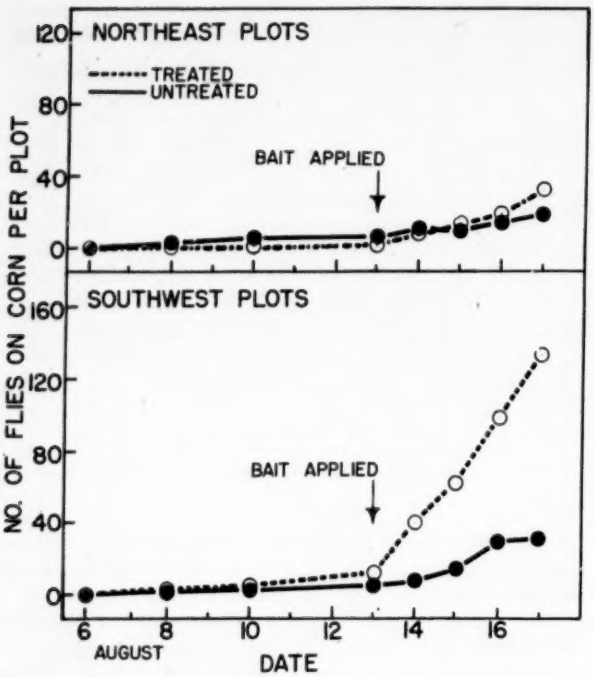

FIG. 2.—Cumulative number of melon flies observed on untreated and yeast hydrolysate treated corn plants. Arrows indicate date of yeast hydrolysate application.

cept the check plots. The fly counts were then continued for another 4 days.

The data presented in figure 2 show that the response to baits varied with the section of the field. In the northeast plots there was no significant difference in fly population between treated and check plots either before or after spraying yeast hydrolysate, an indication that yeast hydrolysate had no effect in inducing fly movement into the sprayed plots. However, in the southwest plots where there was a marked increase of flies in the treated plots it is evident that there was fly movement into the plots sprayed with yeast hydrolysate. This increase, however, does not mean that the flies moved from the untreated to the treated plots for there was also a less marked but a noticeable increase even on the untreated plots. Apparently flies from outside the experimental areas moved into this section of the field and that a higher number remained on the treated than untreated plots.

Movements from non-host to host plants.—Two experiments were conducted to determine whether the application of yeast hydrolysate induces fly movement from untreated non-host plants to yeast hydrolysate-treated host plants. The first was conducted on a $\frac{1}{4}$-acre cucumber field surrounded by two rows of corn plants. Two 25-foot corn plots were marked off on each of the northeast and southwest sections of the cucumber field. Adjacent and parallel to these plots and approximately 25 feet from them, cucumber plots 25 feet long were also staked. Counts were taken on the number of melon flies observed within these plots starting at 9:30 A.M. and, after three pretreatment counts, a spray containing 1 pound yeast hydrolysate per 100 gallons was applied at 11:00 A.M. only to the cucumber plants within the 25-foot plots. After treatment, six additional fly counts were taken from each of the plots. The results obtained (figure 3) show that, although flies were present on the corn plants, no fly was observed on the sprayed cucumber plants which were located 25 feet away from the adjacent rows of corn. These data indicate that the yeast hydrolysate spray did not induce movement of flies from the untreated corn to the treated cucumber plants.

The second experiment was conducted in a cantaloupe planting bordered by two rows of corn plants. In five widely separated sections of this field, four hills of corn 25 feet apart were staked out. At 12 and 24 feet away from these hills of corn, four cantaloupe hills each 25 feet apart were also staked out. A spray containing 2 pounds of a 25% malathion wettable powder and 1 pound of yeast hydrolysate per 100 gallons was applied to the marked hills of corn and cantaloupe plants. Three hours after treatment, the number of dead flies on the soil under each of the treated plants was counted. The results, presented in table 3, show that the number of dead flies under the cantaloupe plants was considerably less than under the corn. As in the previous study these data show that comparatively few flies moved on to cantaloupe plants sprayed with yeast hydrolysate.

Movements from untreated to treated half of a field.—The purpose of this study was to determine whether melon flies can be induced to move from one half of a field to the other half by use of yeast hydrolysate bait. A rectangular $\frac{1}{4}$-acre cucumber planting bordered on four sides by a double row of corn was divided into two plots of equal size. Four 40-foot corn and cucumber plots were marked off in various parts of each half of the cucumber field. Pretreatment counts were made by counting the

Table 3.—The number of dead melon flies found three hours after treatment under corn and cantaloupe plants.[a]

Location	Corn Plants		Cantaloupe Plants			
			12 Feet from Corn		24 Feet from Corn	
	Total No. of Flies	No. of Flies per Hill	Total No. of Flies	No. of Flies per Hill	Total No. of Flies	No. of Flies per Hill
1	2	0.5	0	0.0	0	0.0
2	5	1.2	1	0.2	0	0.0
3	12	3.0	1	0.2	0	0.0
4	13	3.2	1	0.2	2	0.5
5	17	4.2	0	0.0	1	0.2

[a] Four replicates treated with malathion-yeast hydrolysate bait spray.

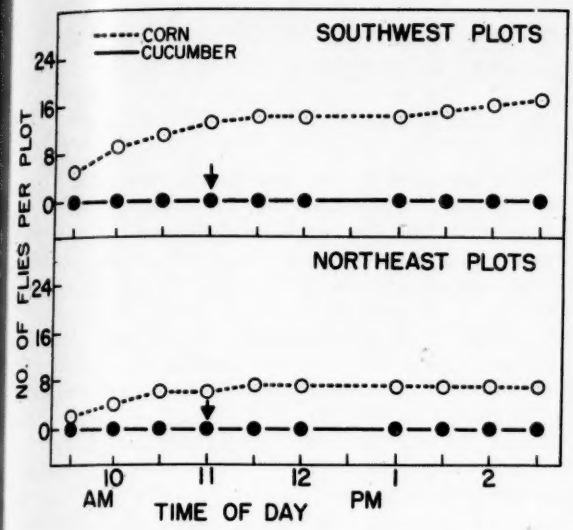

FIG. 3.—Cumulative number of melon flies observed on unsprayed corn plants and on nearby cucumber plants sprayed with yeast hydrolysate. Arrows indicate time when cucumber plants were sprayed.

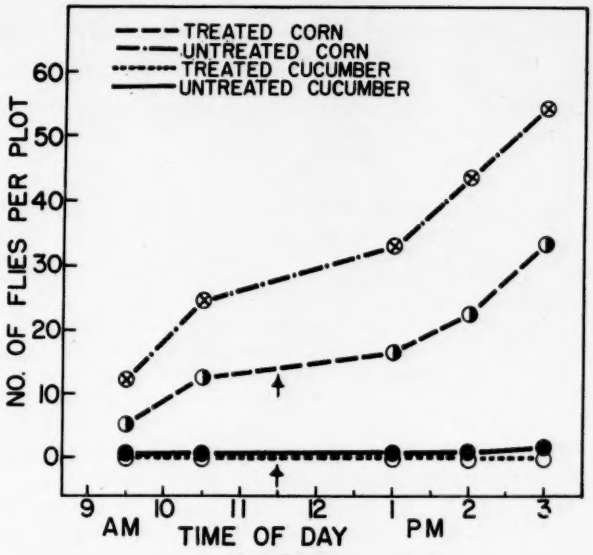

FIG. 4.—Cumulative number of melon flies counted on corn and cucumber plants sprayed with yeast hydrolysate and those which were not sprayed. Arrows indicate the time at which the bait was applied.

numbers of adults on corn and cucumber plots. After two counts, yeast hydrolysate at the concentration of 1 pound per 100 gallons was applied to all of the corn and cucumber plants in one half of the field. After treatment an additional two counts were made.

The data, presented in figure 4, show that there was no movement from one side of the field to the other. The fly counts on treated and untreated corn as well as those on the treated and untreated cucumber plants ran almost parallel. If there were movements from the untreated to the treated half of the field, there should have been an increase in one and a decrease in the other. However, because this change did not occur, it is evident that there was no movement between plots. The parallel rise in fly populations on treated and untreated corn indicated that the flies moved to the respective corn plots from other untreated areas at approximately the same rate.

DISCUSSION.—The results of this study showed variation in the response of the melon fly adults to yeast hydrolysate bait applied in the field. This variation might be attributed to the variation in abundance of flies within localized areas in which these studies were conducted. When the fly population is high, as for example in or near recently abandoned crop areas, the quantity of natural adult food in the form of nectar, honeydew, and glandular exudate available per fly is small, and hence under such an over-populated condition a large number would respond to the bait. However, under conditions of low fly density, the quantity of natural food available per fly is large; hence the low response to the yeast hydrolysate bait. Theoretically, we can expect 100% of the flies responding only when natural foods have been depleted. However, such a situation, except perhaps in small localized areas, rarely occurs under natural conditions.

The response to bait by the melon fly was found to be influenced by the substrate on which the bait was applied, a finding which is indeed surprising in view of the observations that in captivity melon flies will feed readily on yeast hydrolysate placed on almost any substrate. In general, the response to the yeast hydrolysate was greatest when applied to plants favored by the adults. This observation suggests that under field conditions melon flies respond primarily to the plant and secondarily to the bait; otherwise, the response should be the same regardless of the substrate on which the bait is applied.

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Effect of Low Temperatures on Survival of Stem-Mother Aphids in Northeastern Maine[1]

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Abstract

Heavy mortality of the stem mothers of the potato aphid (*Macrosiphum solanifolii* (Ashm.)) and the buckthorn aphid (*Aphis abbreviata* Patch) occurred on the primary hosts in the spring of 1956 following an extended period of subnormal temperatures. Occurring mostly near the end of nymphal development, the mortality likely was due chiefly to subjection of the immature stem mothers to prolonged subnormal temperatures rather than to sharp drops in temperature.

Springtime development of several species of potato-infesting aphids on their primary hosts in northeastern Maine has been followed rather closely during the last 15 years (Shands & Simpson 1954). There have been several indications of an early-season drop in abundance of the potato aphid (*Macrosiphum solanifolii* (Ashm.)) on swamp rose (*Rosa palustris* Marsh.), but this drop was not confirmed definitely until 1956. This paper summarizes the evidence of early-spring mortality of stem mothers of the potato aphid on swamp rose and of the buckthorn aphid (*Aphis abbreviata* Patch) on alder buckthorn (*Rhamnus alnifolia* L'Her.).

Potato Aphid.—Table 1 shows spring population trends of the potato aphid on swamp rose at 12 stations under approximately normal conditions in 1955 as compared with 1956, when early-season abundance of the eggs (Shands *et al.* 1956) and aphids was markedly greater. Hatching began about 10 days earlier in 1956 than in 1955, but the spring peak was several days later, and the aphid was generally much less abundant.

At most stations reduced abundance at the 1956 spring peak appeared to be due chiefly to greater-than-usual mortality of the developing stem mothers during May. The reduction from the May peak averaged 53%. Although the reduction began shortly after the eggs hatched, nearly 60% of it occurred during the last week of May, or just before the first stem mothers matured.

On May 22 and 23 twenty stem-mother nymphs in the second to fourth instars were caged separately on branches of swamp rose at one of the stations. Practically all the nymphs were caged without being moved by enclosing the infested branches in drawstring scrim cages. Subsequent examination revealed that 16 of the nymphs died, most of them shortly before they should have matured in late May. Two of the replacement stem mothers placed singly in these cages on May 29 also died before maturing.

Buckthorn Aphid.—Similar results were obtained from studies of the stem-mother generation of the buckthorn aphid on its primary host. Not a single aphid lived to maturity out of five separately caged as first-instars on May 22, and only three out of 11 fourth-instars caged on May 30. However, among those caged as fourth-instars on or after June 4, the date the first aphids matured,

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[3] Maine Agricultural Experiment Station.

Table 1.—Average numbers of potato aphids on 100 buds[a] of swamp rose at various stations in northeastern Maine, 1955 and 1956.

Date	Station Number												Average
	1	2	3	4	5	6	7	8	9	10	11	12	
1955													
May 5– 6	T[b]	—	0	1	3	—	—	—	—	—	4	—	1.6
10–11	1	1	1	2	2	3	1	2	1	2	3	—	1.7
18–19	1	2	0	6	1	1	T	2	1	1	2	—	1.6
25–27	9	24	11	52	6	33	9	9	68	41	154	—	37.8
June 2– 3	2	31	7	31	7	48	12	15	63	31	152	—	36.3
8– 9	T	58	15	27	12	17	33	10	160	154	162	—	58.9
16–17	6	190	89	146	26	68	118	66	94	351	110	—	114.9
23–24	2	232	204	224	12	83	77	19	1	27	575	—	132.4
30–July 1	1	61	13	8	4	4	14	3	8	1	13	—	11.8
July 7– 8	0	12	0	1	0	0	0	1	1	10	1	—	2.4
1956													
April 26–27	0	—	0	2	—	1	—	0	0	T	1	4	2.0
May 7– 8	T	2	2	7	4	3	0	0	2	1	14	3	3.2
16–17	T	3	1	3	2	4	1	T	2	1	16	3	3.0
23–25	T	3	T	2	1	1	1	1	2	2	15	2	2.5
31–June 1	0	1	T	2	7	T	T	T	T	3	5	T	1.5
June 7– 9	0	6	7	14	5	13	0	T	3	2	43	0	7.8
14–15	0	6	4	13	3	11	2	0	3	19	109	0	14.2
21–23	1	58	3	31	17	13	1	T	40	34	1034	—	102.7
28–29	0	82	36	30	3	17	12	0	41	23	1331	1	131.3
July 6– 7	0	4	6	3	0	13	1	0	3	6	215	0	20.9
13	—	8	1	—	0	0	0	0	0	0	22	0	2.8

[a] A bud includes all the new growth arising from it.
[b] Less than 0.5.

Table 2.—Air temperatures (° F.) at 4 feet above ground in the shade at Aroostook Farm, Presque Isle, Maine, April 17 to June 4, 1956.

DATE	MAXIMUM	MINIMUM	WEEKLY AVERAGE
April			
17	50	38	
18	47	33	
19	44	32	
20	47	32	38.5
21	49	24	
22	43	28	
23	44	28	
24	47	28	
25	46	28	
26	52	28	
27	57	32	40.1
28	52	36	
29	48	28	
30	47	32	
May			
1	51	26	
2	55	25	
3	53	35	
4	49	35	40.8
5	57	30	
6	56	35	
7	44	20	
8	46	23	
9	55	25	
10	52	39	
11	62	24	45.0
12	60	39	
13	64	50	
14	60	31	
15	63	38	
16	62	42	
17	47	32	
18	54	28	45.3
19	57	36	
20	52	33	
21	60	30	
22	72	34	
23	71	36	
24	43	27	
25	53	25	45.1
26	60	35	
27	58	35	
28	47	35	
29	71	33	
30	68	49	
31	63	52	
June			
1	77	56	62.0
2	75	56	
3	75	61	
4	73	59	

virtually all lived and matured. In other words, as with the potato aphid, mortality was rather heavy just before maturity.

OTHER APHIDS.—We have no observations of low-temperature effects upon developing stem mothers of other potato-infesting aphids, but indirect evidence suggests there may be some in the green peach aphid (*Myzus persicae* (Sulz.)) on Canada plum (*Prunus nigra* (Ait.)). Because of the more protected environment, these effects may be of considerably less importance for the foxglove aphid (*Myzus solani* (Kltb.)) on hawkweed (*Hieracium* spp.).

DISCUSSION OF RESULTS.—Our results do not agree fully with those of Peterson (1918) or Headlee (1919). Peterson observed mortality of the apple grain aphid *Rhopalosiphum fitchii* (Sand.)). when the young stem mothers were subjected to temperatures low enough to frost the young apple leaves. Headlee said that outbreaks of aphids in New Jersey probably were prevented because of heavy mortality among colonies due to sharp drops in temperature below the freezing point. In northeastern Maine no appreciable mortality of the potato aphid was observed immediately when the temperature dropped from a high of 56° F. on May 6 to a low of 20° on May 7 (tables 1 and 2). On at least one occasion we have observed survival of very young potato aphid nymphs on swamp rose following a minumum of about 16°.

Rather than sharp drops in temperature, generally low temperatures throughout the period of their development may have been primarily responsible for the heavy mortality of potato and buckthorn aphids in 1956 (table 2). Daily means were generally much below normal during the period April 18, when hatching of the potato aphid began, to May 29 when the first stem mothers matured on swamp rose. During May the monthly mean temperature at Presque Isle was 5.3° F. below normal. The low temperatures lengthened the development time of the potato aphid from about 3 weeks to 6 weeks. Because of this prolonged interval, they may have been more susceptible to the low minima on May 24 and 25.

Another factor possibly affecting survival at low temperatures was the quality and quantity of the foliage as food for the nymphs. On 37 days between April 17 and June 1 the minimum air temperature at 4 feet above ground level was 36° F. or lower, and on 19 of these days it was below 32°. On several occasions rose foliage was noticeably frosted, and its rate of growth generally was much retarded throughout April and May. A similar but less pronounced effect upon foliage of alder buckthorn was observed throughout northeastern Maine during the same period.

These observations show that mortality of developing stem mothers from unfavorable spring weather must be taken into account in any attempt to predict abundance of the spring migrant potato and buckthorn aphids.

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Pollination of the Passion Fruit in Hawaii[1]

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ABSTRACT

Preliminary observations made prior to this study showed that in certain areas the passion fruit (*Passiflora edulis* f. *flavicarpa* Degener) plants did not set fruit satisfactorily. The present study indicated that the failure to set fruit was due to blossom rather than immature fruit drop. The percentage of blossom drop was found to vary from 21.6 to 64.4%. The passion fruit, a plant with perfect flowers, is self-sterile and insects are the principal pollinators. Among the 15 insect species found to be associated with the flowers, the carpenter bee (*Xylocopa varipuncta* Patton) and the honey bee (*Apis mellifera* L.) are the most important pollinators. The efficiency of pollination by natural pollinators acting together varied with locality. Lack of fruit set was found to be due not only to lack of pollination but also to other unidentified factors.

The yellow passion fruit or yellow-fruited lilikoi, *Passiflora edulis* f. *flavicarpa* Degener, was introduced into Hawaii from Australia in 1923 (Pope 1935). Since then it has been grown in experimental plots of the Hawaii Agricultural Experiment Station, in backyards, and in small commercial plantings. Recently there has been a revival of interest in the commercial production of this fruit as one means of expanding the economic base of the Territory. To be of assistance to the passion fruit industry, investigations on the entomological problems of the passion fruit were initiated (Nishida 1954). Preliminary studies conducted during the course of these investigations showed that lack of pollination and fruit fly attack on young fruits were some of the causes of poor fruit set. In addition, subsequent observations showed that fruit drop may also be caused by the larvae of an anthribid beetle, *Araecerus vieillardi* (Mont.), which feed on the peduncle. To date this damage, which occurs usually on nearly mature fruits, has been observed only at Waimanalo, Oahu (Nishida 1957).

The problem of unsatisfactory fruiting of the passion fruit, a plant with perfect flowers, has long been recognized. Pope (1935) ascribed the unsatisfactory fruiting behavior to lack of pollination caused by (1) unsatisfactory environment and (2) protandrous habit; *i.e.*, the pollen ripening before the stigma is receptive to the pollen. In regard to unfavorable environment Pope observed that when this crop was grown at 100 to 1200 feet elevation the plant made vigorous growth, but rarely produced high fruit yields. The heaviest yields were obtained near sea level. It is not known whether the poor yields obtained were due to lack of pollination or to other factors.

Although recognized in the past, the problem of passion fruit pollination has not been investigated to any great extent. Pope (*op. cit.*) reported that certain kinds of pollen carriers, viz., the carpenter bee (*Xylocopa varipuncta* Patton), large moths, and humming birds, are of such size that they were capable of transferring pollen from the stamens to the stigma by carrying pollen adhering to their bodies. It was stated, however, that although carpenter bees were frequently seen visiting the passion fruit flower, moths were rarely seen and that humming birds do not occur in Hawaii. In addition to the carpenter bee, Pope believed that under dry sunny conditions, winds aided in the transfer of pollen from the stamens to the pistils of the same flower. However, this type of pollen transfer is of no importance in view of the recent finding that the passion fruit is self-sterile to a very high degree (Akamine *et al.* 1954, Akamine & Girolami 1957). Furthermore, wind pollination does not appear to be of importance in cross pollination because of the large, heavy, and sticky pollen grains which are not readily disseminated by winds. By use of pollen traps it was shown by Akamine & Girolami (1957) that the number of pollen grains carried by air currents was very small and hence pollination by winds was not important. On the other hand, there are features which suggest strongly that pollination is accomplished by insects. These are (1) large attractive flowers, a characteristic of many insect pollinated flowers, (2) strong odor which permeates throughout the field during the blooming period, (3) abundance of nectar, and (4) large sticky pollen grains. The presence of a number of nectar and pollen feeding insects on the open flowers also suggested the importance of insects as pollinators.

EXTENT OF FLOWER AND IMMATURE FRUIT DROP.—Two studies were conducted to determine the extent of flower and immature fruit drop. In the first study the number of flowers and immature fruits that had fallen to the ground were counted. These data gave information on the stage of development in which fruit loss occurred. In the second study terminal twigs, which bear the flowers and fruits of the current season, were selected at random and the number of fruits that had set, as well as the number that had dropped were counted. The number of flowers or immature fruits that had dropped off may be readily counted because the abscission occurs near the base of the flower leaving a long peduncle which remains on the plant for a long time.

The data obtained on flower and immature fruit drop as determined by counting the number of flowers and immature fruits that had dropped to the ground, presented in table 1, show that the number of flowers that dropped to the ground was considerably higher than that of immature fruits. These data indicate that unfruitfulness is due largely to flower rather than immature fruit drop. It is also evident that the melon fly (*Dacus cucur-*

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Table 1.—Flower and immature fruit drop of the passion fruit as determined at Waimanalo, Oahu.[a]

Location	Number of Flowers	No. Immature Fruits		Total
		Stung[b]	Not Stung	
1	152	2	0	154
2	117	1	4	122
3	203	1	3	209
4	95	6	4	105
Mean	141.7	2.5	2.7	147.5

[a] Number of flowers and fruits on the ground under 24 linear feet of row were recorded at each location.

[b] Oviposition puncture made by the melon fly, *Dacus cucurbitae* Coq., and the oriental fruit fly, *Dacus dorsalis* Hendel.

bitae Coq.) and the oriental fly (*Dacus dorsalis* Hendel), which puncture the fruits after they have set, contributed little to the overall unfruitfulness.

The data on flower drop, obtained by examining the terminal shoots on which the flowers and fruits of the current season are borne, are shown in table 2. The flower drop, which includes a small percentage of immature fruit drop, ranged from 21.6 to 64.4% with the highest percentage at Pupukea and the lowest in Manoa Valley.

FLOWER VISITING INSECTS.—The insects associated with the passion fruit flowers are listed in table 3. Species that were most frequently and consistently associated with the flowers were the carpenter bee, *Xylocopa varipuncta*, and the honey bee, *Apis mellifera* L. The hover fly, *Eristalis arvorum* (Fab.), and the oriental blow fly, *Chrysomya megacephala* (Fab.), were also observed on passion fruit flowers, but they were observed less frequently than either the carpenter or the honey bee. Other insects listed in table 3 were not commonly observed on passion fruit flowers.

Data on relative abundance, taken only on species commonly associated with the passion fruit flower, were obtained by walking between the passion fruit rows and tallying each individual observed by use of a laboratory multiple counter. The results obtained (table 4) indicate that although their relative abundance varied with locality, the carpenter bee and the honey bee were the most abundant. The carpenter bee and the honey bee were the most abundant species in the Waimanalo and Manoa areas of Oahu but the honey bee was the most abundant in the Kunia areas of Oahu and also in the Hilo areas of Hawaii.

Table 2.—The extent of flower drop in various localities of Oahu in 1956 as determined by examining terminal vines which produce fruits.

Locality	Sampling Date	Number of Samples[a]	Mean No. of		Mean Per Cent Drop
			Fruit Set Possible per Sample	Flower Drop per Sample	
Waimanalo	May 6	4	45.5	22.0	46.5
Waimanalo	June 6	6	34.8	14.0	41.1
Pupukea	May 6	4	27.5	17.7	64.4
Manoa	May 20	5	23.2	5.0	21.6
Waialua	May 6	6	34.5	14.0	38.5

[a] Each sample consisted of five terminal vines selected at random.

Table 3.—Insects associated with passion fruit flowers and their relative abundance.

Species	Frequency of Occurrence
ORTHOPTERA	
Conocephalus saltator (Sauss.)	Rare
THYSANOPTERA	
Thrips hawaiiensis f. imitator Pr.	Frequent
COLEOPTERA	
Conotelus mexicanus Murray	Rare
HYMENOPTERA	
Apis mellifera L.	Very frequent
Iridomyrmex humilis Mayr	Rare
Pheidole megacephala (Fab.)	Rare
Xylocopa varipuncta Patton	Very frequent
DIPTERA	
Chyromya sp.	Rare
Chrysomya megacephala (Fab.)	Frequent
Dacus cucurbitae Coq.	Frequent
D. dorsalis Hendel	Frequent
Desmometopa sp.	Rare
Eristalis arvorum (Fab.)	Frequent
Euxesta quadrivittata (Macq.)	Frequent
Ophyra nigra (Wied.)	Rare
Milichiella lacteipennis Loew.	Rare
Volucella obesa (Fab.)	Frequent

EFFICIENCY OF THE POLLINATORS.—The efficiency of the pollinators was estimated in the field by selecting at random fully developed buds or newly opened flowers and then tagging, hand pollinating, and bagging them according to the following procedure: A, flowers tagged and bagged; B, flowers tagged, hand pollinated, and bagged; C, flowers tagged and left without bagging; D, flowers tagged, hand pollinated, and left without bagging. The purposes of these procedures were (1) to determine whether self-pollination was possible (procedure A), (2) to determine the maximum fruit set potentially possible when pollination was adequate (procedure B), (3) to determine the extent of pollination by natural agencies (procedure C), and (4) to determine whether covering had any adverse effect on fruit set (precedure D). The data obtained from procedure D showed that covering

Table 4.—The relative abundance of the carpenter bee, honey bee, hover fly, and the oriental blow fly in passion fruit fields.

Date (1956)	Locations	Mean Number per 100 Feet Row				Relative Abundance of Flowers in Bloom
		Carpenter Bee	Honey Bee	Hover Fly	Oriental Blow Fly	
	Hilo, Hawaii					
Nov. 14	A	0.0	3.8	1.9	1.9	Few
Nov. 15	B	0.5	5.1	2.0	0.0	Few
Nov. 15	C	0.4	2.3	0.0	0.0	Few
Nov. 17	D	0.0	4.5	1.9	0.0	Few
Nov. 19	E	0.2	4.7	1.1	0.2	Few
	Kunia, Oahu					
June 20	A	0.0	53.4	0.0	0.0	Abundant
June 20	B	0.0	70.0	0.0	0.0	Abundant
	Waimanalo, Oahu					
July 12	A	12.0	0.0	3.0	0.0	Abundant
July 12	B	5.0	15.0	1.7	0.0	Abundant
July 12	C	2.5	7.5	0.0	0.0	Abundant
June 13	Manoa, Oahu	1.0	11.5	3.5	0.0	Abundant
June 21	Manoa, Oahu	11.3	0.9	3.6	0.0	Abundant

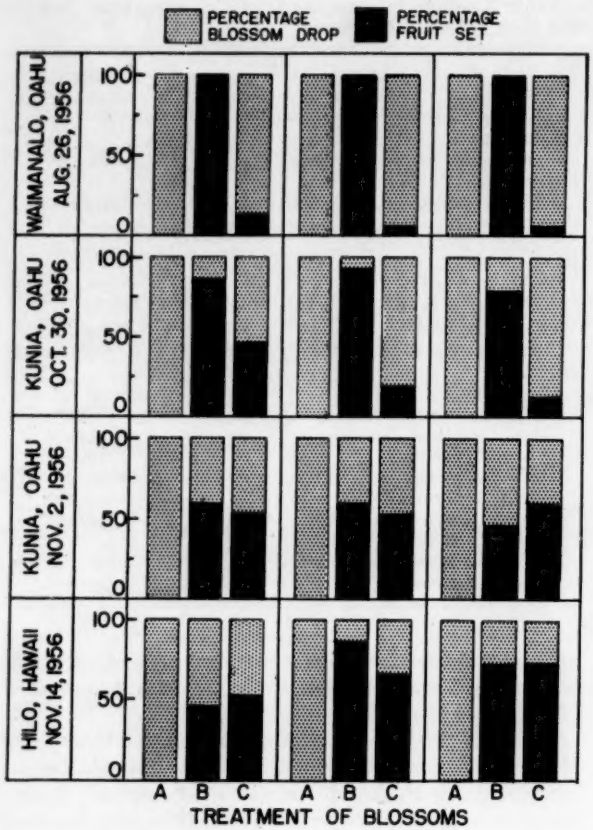

FIG. 1.—The efficiency of the pollinators of passion fruit flowers as determined by tagging and bagging techniques: A, blossoms bagged; B, blossoms hand pollinated and bagged; and C, blossoms tagged and left unbagged.

had no adverse effect on fruit set and are not included in the data presented.

In hand pollination the following procedures were used. The anthers were obtained from a plant other than the one bearing the flowers which were to be pollinated. Although not always possible because of insufficient number of flowers, the anthers were obtained in each test from a single plant. These procedures were used because previous observations had indicated that the passion fruit is self-sterile and also cross incompatible among certain strains. (Akamine *et al.* 1956). In pollinating, a liberal amount of pollen was applied to each of the stigmatic lobes of the unemasculated flower by gently rubbing the anthers against the stigma. The flowers were examined 3 to 7 days later during which time those with unfertilized ovaries turned yellow and dropped off, but those fertilized remained on the plant.

Otter Kraft paper bags were used in bagging the flowers. After placing a bag over the flower, it was sealed by a hand stapler. A few tests were conducted with polyethylene bags. These bags were not satisfactory because of the excessive accumulation of moisture within them. Fruit set was very poor when flowers were covered with polyethylene bags even when small holes were made to take care of the excessive moisture.

An evaluation of the efficiency of pollination by natural agency may be made by comparing the percentage of pollination obtained by hand pollination and that obtained by natural agency. If the natural pollinators were efficient there should be only a small discrepancy between the values obtained by these two procedures and conversely, a wide discrepancy when the pollinators were inefficient. From the data presented in figure 1 it may be seen that the efficiency of natural pollinators varied with locality. The lowest efficiency was encountered at Waimanalo, Oahu, and the highest at Kunia, Oahu (November 2, 1956), and at Hilo, Hawaii.

The data presented in figure 1 show other features concerning passion fruit pollination. When the flowers were bagged without emasculation and hand pollination (Procedure A) there was no fruit set. This failure to set fruit indicates that the passion fruit flowers are not self-pollinating, a corroboration of the results reported previously (Akamine *et al.* 1956). Another feature indicated by the data is that the maximum fruit set potentially possible varied with locality. At Waimanalo this maximum was 100%, but for some unknown reason it was lowest at Kunia (November 2, 1955). The failure to obtain maximum fruit set even among hand-pollinated flowers indicates that poor fruit set is in part due to causes other than lack of pollination.

DISCUSSION.—On the basis of this study it is not possible to draw definite conclusions as to which insects are the most effective pollinators of the passion fruit because the technique used in this study measures an overall efficiency of all pollinators taken together. A general idea as to the importance of different species may be obtained however on the basis of the frequency at which a species visits the flowers and their general abundance. Considered on this basis, the carpenter bee and the honey bee are without doubt the most important pollinators.

The efficiency of the pollinators was found to vary in the different areas where these studies were conducted. Such variations may result from a number of factors; viz., (1) differences in the general abundance of the pollinators in relation to the abundance of passion fruit flowers, (2) lack of synchronization between blooming period and pollinator activity, and (3) abundance of other flowers which might be highly favored by the pollinators. An evaluation of the importance of these factors as well as others would be of considerable value in an undertaking in which the activities of the pollinators are manipulated by man in order to obtain satisfactory pollination.

The maximum fruit set possible was also found to vary with locality. Evidently such variations were also observed by Pope (1935) for he stated that in certain areas the fruits failed to set even with hand pollination. To be sure, some of these variations may be due to cross incompatibility, a type of sterility of the passion fruit plant reported by Akamine *et al.* (1956).

The data obtained in this study showed that in certain areas maximum yields were not being obtained by growers because of the flower drop caused by lack of pollination. In order to obtain maximum yields the practice of encouraging pollinators is much to be desired. Furthermore, in certain areas where the population of natural pollinators is limited, it seems unwise to expand passion fruit acreages beyond the pollinating capacity of the pollinators present in the area in question. If the passion fruit industry is going to expand, a study of the insect pollinators of the passion fruit in other countries, prefer-

ably in its native habitat, and the importation of pollinators into the Territory are projects worthy of consideration.

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The Treadle Sprayer and the Cable-Type Back Rubber for Control of Biting Flies on Cattle in Pennsylvania[1]

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ABSTRACT

The effectiveness of various insecticide-repellent formulations applied by means of self-treating devices for the control of the horn fly, *Siphona irritans* (L.), and the stable fly, *Stomoxys calcitrans* (L.), on cattle under Pennsylvania conditions was tested during 1955 and 1956.

A spray containing 50% butoxy polypropylene glycol and 5% methoxychlor in methylated naphthalenes was tested during 1955 with a treadle sprayer and a cable-type back rubber. With the treadle sprayer, an average of 90.0% control of the horn fly and 34.7% control of the stable fly resulted when one round-trip application was made daily. Because of the excitement and nervousness caused among the animals, approximately one-third of the animals going through the sprayer received only partial treatment or none at all. The back rubber installed in a small pasture with a few trees accomplished almost perfect control of the horn fly, but less satisfactory control of the stable fly. A significant decrease in effectiveness was noted when the back rubber was located in a pasture with numerous dead trunks and branches, but no decrease was observed in wooded pastures devoid of dead trunks and branches. Studies of the residual effect showed that control of the horn fly throughout a post-treatment period of 25 days varied from 52.5 to 99.7%. The residual effect against the stable fly lasted for only 15 days, control varying from 33.0 to 54.4%.

In 1956, units of the portable model of the rubbing device, treated with four insecticide-repellent formulations including butoxy polypropylene glycol, methoxychlor, Lethane 384 and synergized pyrethrins, were installed indoors in a basement barn, and in three pastures of different sizes and with different numbers of trees, but relatively free of dead trunks or stumps. All formulations provided effective and consistent control of the horn fly. No significant difference in control was found among the four formulations tested under the different conditions. It was noted that 5% methoxychlor in diesel oil provided excellent control of the horn fly and that the addition of butoxy polypropylene glycol did not significantly enhance the effectiveness of methoxychlor.

None of the four formulations tested provided satisfactory control of the stable fly and there was considerable variation in the effectiveness of each formulation.

None of the materials tested on the back rubber had any apparent adverse effects on the animals used in the experiments.

The cable-type back rubber has been used for combating insect pests on cattle in different states including Pennsylvania. Since it was first described by Rogoff & Moxon (1952), researchers in different parts of the country have conducted tests to determine its effectiveness for the control of biting flies and lice on both beef and dairy cattle. It has been found to privide good protection against horn flies by Rogoff & Moxon (1952) in South Dakota, Lindquist & Hoffman (1954) in Oregon, Raun & Casey (1956) in Iowa and Goodwin (1956) in South Carolina. It has also been demonstrated to give effective control of cattle lice by Hoffman (1954) in Oregon, Neel (1956) in Mississippi and Gressette & Goodwin (1956) in South Carolina. The reports have indicated that the device has failed to give satisfactory control of stable flies and horse flies.

The treadle sprayer was first used by Bruce & Decker (1951) against tabanids on range beef cattle. They reported that a higher degree of control was obtained with this device than with other kinds of applicators used on beef or dairy cattle. Bruce (1952) stated that a concentrated solution of activated pyrethrins employed in a treadle sprayer gave practical control of horse flies and other biting flies on cattle in Illinois. Granett *et al.* (1955) found that the sprayer was effective in controlling biting flies in New Jersey; best results were obtained when cattle received three or four round-trip applications of synergized pyrethrins per day. Bruce & Decker (1955) reported that results obtained by users of treadle sprayers in many parts of the country varied from phenomenal success to dismal failure. The authors listed a number of factors affecting the performance of the treadle sprayer; animal routine was considered the most important among the factors mentioned.

The objectives of the investigations described in this paper were to test and compare the effectiveness of the treadle sprayer and cable-type back rubber for the control of the horn fly, *Siphona irritans* (L.), and the stable fly, *Stomoxys calcitrans* (L.), under Pennsylvania conditions, and to determine the effectiveness of various ma-

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terials applied with back rubbers installed in different locations and surroundings, both indoors and outdoors.

METHODS AND MATERIALS.—The experiments were conducted during 1955 and 1956 on the farms of the Pennsylvania State University at University Park.

(1) The treadle sprayer: The principal parts of the treadle sprayer (Yellow Devil Treadle Sprayer Model 69, manufactured by Engine Parts Manufacturing Company, Cleveland, Ohio.) consisted of a treadle and an upright on which were mounted an air pump and two nozzles each connected to a jar containing spray material. During operation, the treadle was placed on the ground in the center of a chute, while the pump, the nozzles and the spray containers were outside the chute. The chute and the sprayer were covered with canvas. When an animal stepped on the treadle, its weight operated the pump, which forced the spray out of the nozzles onto one side of the animal. As the animal made its return trip through the chute, its other side was sprayed. A livestock spray, formulation I, containing 50% butoxy polypropylene glycol and 5% methoxychlor in methylated naphthalenes, was used to treat a herd of 14 dairy Holsteins. A second herd of 10 untreated dairy Holsteins was used as a check.

Prior to treatment, the animals were made to familiarize themselves with the operation by making a round-trip through the sprayer once or twice daily for 2 weeks, after which spraying tests were begun. As a rule, applications were made early in the morning. One round-trip application was given each animal per day. Each of the two nozzles delivered 0.5 ml. per stroke of the pump and each animal received approximately 4 ml. per round-trip application. A total of 21 applications were made. After spraying, treated and untreated animals were confined in adjacent pastures and fly populations were counted at regular intervals of 1 and 2 hours with the aid of field glasses. The mean number of flies per animal was determined by counting the flies on 10 animals in each group.

(2) Cable-type back rubber: The device used in 1955 was made of a log chain and barbed wire wrapped with burlap sacks. The upper end of the chain was tied around a tree and the lower end fastened to a ground anchor. The device was modified in 1956 so that the lower end of the chain was connected to a spring which was clamped to an eye-bolt embedded in the middle of a rectangular concrete block 8×8×48 inches in size. The block was also wrapped with burlap sacks soaked with the insecticide and was held in place by several small wooden stakes (fig. 1). This arrangement made it possible to move the rubbing unit conveniently from one pasture to another as necessary and to install it wherever the cattle loafed or gathered most frequently.

In the summer of 1955, five herds of cattle were used in the tests. Three herds, with 10 dairy Holsteins in each, were placed in three adjacent pastures (A, B and C) which varied in size from 2 to 7 acres. The arrangement of the three pastures is shown in figure 2. The back rubber was installed in pasture A, consisting of 2 acres with six scattered trees. In the beginning of the test, herd No. 1, heavily infested with flies, was permitted access to the back rubber in pasture A for 48 hours. Being nearly free of flies at the end of 48 hours, they were transferred to pasture B for studies of the residual effect. Herd No. 2 was then driven into pasture A for self-treatment, and herd No. 3 was driven into pasture C to serve as check animals. The three herds were rotated clockwise twice during 8 weeks of observations. The first rotation took place 15 days after the observations began and the second rotation was made 25 days after the first rotation. Thus, during the period covered by the experiment, each herd served as treated, check, and post-treatment animals for studies on the residual effect. The results obtained from the three herds in turn in each of the pastures were combined and averaged. In an attempt to study the influence of the size of the pastures, number of trees and other competing objects, herd No. 4 with 30 head of beef cattle including Aberdeen Angus and Shorthorns were allowed to use a rubbing unit installed in pasture D. This pasture covered 10 acres with approximately 130 trees and 40 dead tree trunks and branches. A similar herd, No. 5, was placed in a nearby pasture to serve as the check.

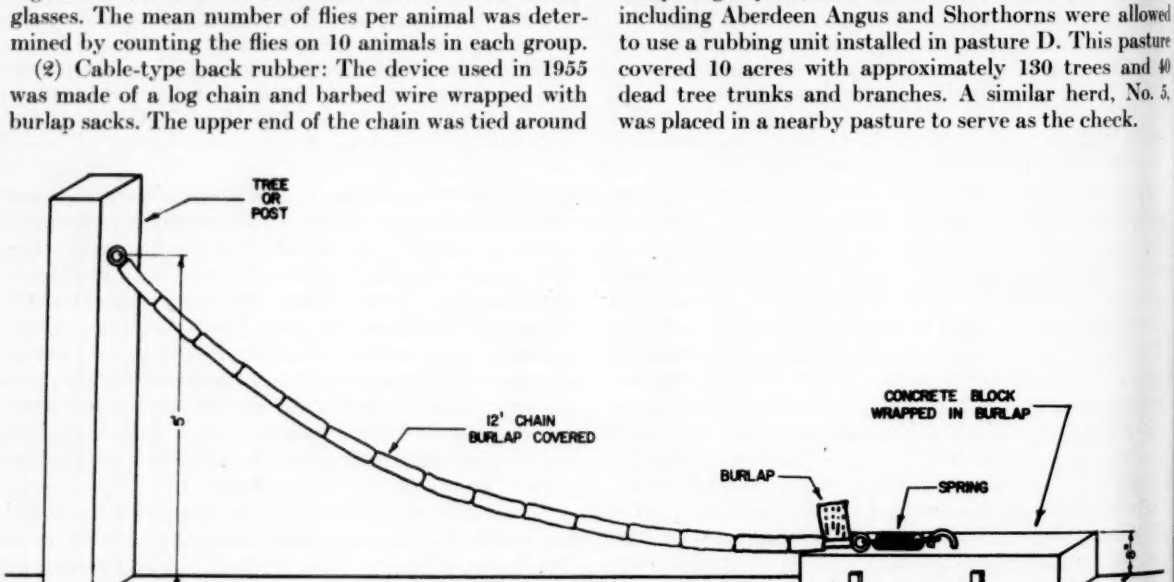

FIG. 1.—A portable cable-type back rubber used in tests on the control of biting flies on cattle in Pennsylvania. University Park, Pennsylvania. 1956.

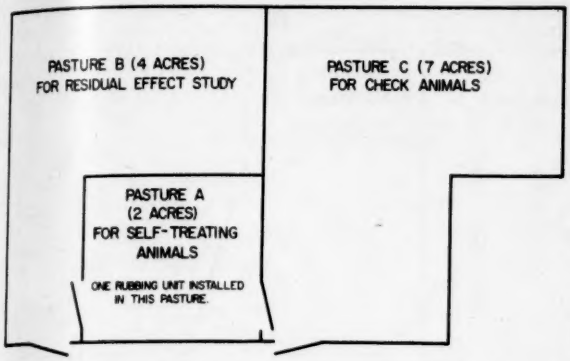

FIG. 2.—Arrangement of pastures in which herds No. 1, 2 and 3 were rotated during tests on the use of cable-type back rubber for biting fly control. University Park, Pennsylvania. 1955.

Daily fly counts were made throughout the observation period, with the exception of Sundays and several rainy days. The fly populations on 10 animals in each group were counted and averaged to give the mean number of flies per animal per day.

During the summer of 1956, tests on the back rubber were conducted to study the effects, if any, on the efficiency of the device due to differences in locations and surroundings, with particular reference to the size of pastures and number of trees in them. Four dairy herds (Nos. 6, 7, 8 and 9) with a total of 113 head were used in the tests. Each herd was divided into two groups similar in breed. One group served as treated animals and the other as the check. The two groups were placed in comparable pastures a short distance apart.

Four insecticide-repellent formulations, I, II, III and IV, were employed. Their principal ingredients were as follows: I. 50% butoxy polypropylene glycol and 5% methoxychlor in methylated naphthalenes; II. 8.6% butoxy polypropylene glycol, 1% methoxychlor, 0.6% Lethane 384, and 0.03% synergized pyrethrins in petroleum distillate; III. 5% methoxychlor in diesel oil; IV. 10% butoxy polypropylene glycol and 4% methoxychlor in diesel oil.

In order to study the feasibility of using the back rubber indoors, a unit was installed in a basement barn. Each of the two groups in herd No. 6, used in this experiment, consisted of 12 heifers of Jerseys, Ayrshires, Holsteins and Brown Swiss. The barn was divided by a partition, each half opening to a separate pasture. The group placed in the side with a back rubber served as treated animals and the group in the other side as the check. The back rubber was treated with formulation I. After 25 days of observation, the rubbing unit was removed and a period of 6 days was allowed for the population of flies on the treated animals to build up to about the same level as on the untreated group. When it was established that the residual effect of the formulation had completely disappeared, a new rubbing unit was installed and treated with formulation II.

The three remaining herds were tested in different pastures to study the effect of pasture size and number of trees on the efficacy of the rubbing device. Herd No. 7 was composed of 22 Holsteins, of which 12 served as treated animals and 10 as checks. The treated group was located in pasture A, previously described, and given access to a rubbing unit soaked with formulation III. The check animals were placed in a nearby pasture. Herd No. 8 was made up of 25 Holsteins, 15 being included in the treated group and 10 in the check. Pasture E, in which the treated group was kept, covered 7 acres with approximately 400 trees located in a strip across the center of the pasture, forming a heavily wooded elongated area. A rubbing unit treated with formulation IV was installed at the edge of the wooded area. The untreated animals were placed in an adjacent pasture. Herd No. 9 consisted of 42 cows of different breeds. The treated group was made up of 30 Ayrshires, Holsteins and Jerseys, and the untreated group contained 12 cows of the same three breeds. The treated group was located in pasture F, consisting of 40 acres with about 40 trees scattered along a fence. The back rubber was attached to one of the trees close to the fence and was soaked with formulation IV. The untreated group was placed in an adjacent pasture.

Observations on both the treated and check animals were made daily except during several days of unfavorable weather. The duration of observations on the different herds varied from 25 to 65 days. The fly populations on a random sample of from 8 to 10 cows were counted and the mean number of flies of each species per cow was determined for each observation. Only populations of the horn fly and stable fly were included in the data, because horse flies occurred in very small numbers.

RESULTS AND DISCUSSION.—Tests made in 1955 showed that, using the treadle sprayer, formulation I, which contained 50% butoxy polypropylene glycol and 5% methoxychlor, gave from 82.9 to 94.7% control of the horn fly, and from 6.4 to 59.6% control of the stable fly, when fly populations were counted between 1 and 7 hours after spraying (table 1). In figure 3a, the curve showing the per cent control of the horn fly indicates that the spray was consistently effective and the decrease in its efficacy, during the 7 hours after spraying, was relatively gradual and slight. On the other hand, the curve for stable fly control shows that the spray was much less effective and the per cent control dropped abruptly 4 hours after spraying. It was noted that one

Table 1.—Control of the horn fly and stable fly on cattle with formulation I, containing 50% butoxy polypropylene glycol and 5% methoxychlor, applied by a treadle sprayer.[a] University Park, Pennsylvania July-September, 1955.

Hours After Spraying	Mean No. of Horn Flies per Animal[b]		Per Cent Control	Mean No. of Stable Flies per Animal[b]		Per Cent Control
	Treated	Check		Treated	Check	
1	3.3	62.6	94.7	3.0	7.3	59.0
3	5.0	70.8	92.8	4.2	10.4	59.6
4	8.8	99.5	91.2	5.1	5.7	10.6
6	10.5	108.7	90.4	3.8	4.8	20.8
7	15.2	88.8	82.9	7.3	7.8	6.4
Average (7-hr.)	8.6	86.1	90.0	4.7	7.2	34.7

[a] Twenty-one round-trip applications.
[b] Average number of flies on 10 animals counted in each of the treated and check group.

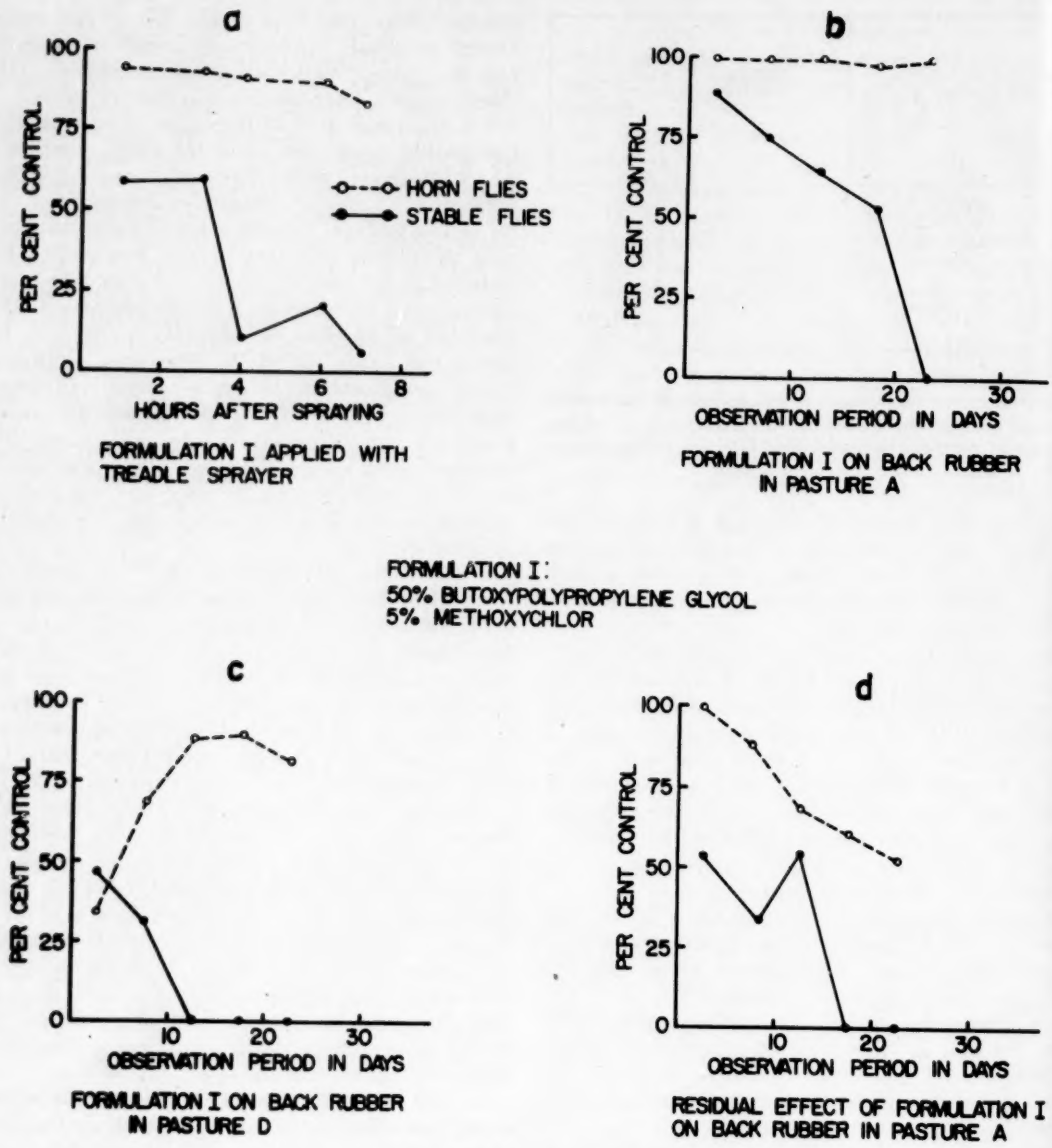

FIG. 3.—Effectiveness of formulation I applied with the treadle sprayer and cable-type back rubber for the control of biting flies on cattle. University Park, Pennsylvania. 1955.

difficulty associated with the use of the treadle sprayer was the excitement and nervousness caused among the animals. Even after 2 weeks of training, some animals jumped as they passed through the sprayer and some stepped on the treadle before the one ahead stepped off, thus interfering with the proper operation of the spraying device. Results of 364 tests conducted over a period of nearly 4 weeks showed that only two-thirds of the animals passing through the sprayer got full treatment (each side of the animal receiving four squirts from the two nozzles), while most of the remainder received half treatment and a small number received no treatment (table 2). This probably was one of the factors responsible for the unsatisfactory control of the stable fly.

In the tests on back rubbers, daily fly population counts taken from each group of animals were combined and averaged for every 5-day period in order to reduce daily variations. Results showed that formulation I, applied with a back rubber, gave almost perfect control of the horn fly in pasture A, which consisted of 2 acres with six trees scattered in it, but the same formulation was less effective when applied on a rubbing unit installed in pasture D. The latter covered 10 acres, with about 130 trees and 40 dead tree trunks and branches. An examination of table 3 shows that, throughout the observation period of 25 days, the per cent control of the horn fly was consistently high in pasture A, varying from 97.5 to 99.9. In pasture D, however, horn fly control averaged only 34.3 and 68.6% during the first and second 5-day periods. It then rose to 88.0 and 89.5% during the third and

Table 2.—Results of tests[a] on the operation of the treadle sprayer.

Treatment	Direction through Sprayer	Per Cent Animals Receiving Indicated Treatment	Average, Per Cent
Full[b]	Entering stable	70.0	66.5
	Leaving stable	63.0	
Half	Entering stable	27.4	28.0
	Leaving stable	28.5	
None	Entering stable	2.6	5.5
	Leaving stable	8.5	

[a] Based on 364 round-trips made by 14 dairy Holsteins.
[b] Each side of the animal receiving four squirts from the two nozzles.

fourth periods and finally dropped to 81.4% during the last 5 days. These results, graphically shown in figure 3b and 3c, clearly indicate that most of the animals in pasture D started self-treatment much later and used the back rubber less frequently than those in pasture A. The difference between the degrees of horn fly control in the two pastures was significant at the 5% level. It was not clear, at this point, whether the difference was due to the presence of dead trunks and branches and/or other factors, such as the size of the pasture and number of trees. However, tests made in 1956, which will be discussed later in the paper, seemed to indicate that in pastures which were free of dead trunks, stumps or branches, neither the size of the pasture nor the number of trees had a significant bearing on the efficiency of the rubbing device for horn fly control. It was assumed, therefore, that the numerous dead tree trunks and branches in pasture D were competing with the rubbing unit and consequently the self-treating device was less utilized by the animals. This assumption was substantiated by the fact that on frequent occasions the animals were seen rubbing on the dead trunks.

It will be noted that formulation I was less effective against the stable fly than the horn fly. In pasture A, the per cent control during 25 days of observation was 88.6 for the first 5-day period, after which it decreased consistently to 0 during the last 5 days. In pasture D, the effect of the formulation was noted only during the first and second 5-day periods, when the fly populations were reduced by 46.4 and 31.8% respectively. The difference between the degrees of control in the two pastures was significant at the 5% level.

A study of the residual effect of formulation I on the three herds self-treated in turn in pasture A showed that after the animals had been denied access to the rubbing device, the per cent control of the horn fly was 99.7, 87.9, 67.9, 60.4 and 52.5 from the first to the fifth 5-day period, respectively. The residual effect against the stable fly was noted only for 15 days, during which the per cent control was 53.0, 33.0 and 54.4 for the first, second and third 5-day period, respectively (fig. 3d).

Results of the experiments in 1956 indicated that all of the four formulations (I, II, III, and IV) tested on the back rubber were consistently effective against the horn fly in different locations, indoors as well as outdoors, but all were less satisfactory for the control of the stable fly. An examination of table 4 shows that formulation I, containing 50% butoxy polypropylene glycol and 5% methoxychlor, utilized by herd No. 6 in a basement barn, gave from 92.3 to 96.7% control of the horn fly, and 54.0 to 87.9% control of the stable fly during the 25-day observation period. Formulation II, which contained 8.6% butoxy polypropylene glycol, 1% methoxychlor, 0.6% Lethane 384, and 0.03% synergized pyrethrins, was used by the same herd for another observation period of the same duration. It provided from 84.5 to 94.6% control of the horn fly, and 32.0 to 88.9% control of the stable fly. Formulation III, containing 5% methoxychlor in diesel oil, was used by herd No. 7 located in pasture A. During the 65-day observation period, horn fly control achieved by this formulation varied from 89.7 to 98.8%, and stable fly control from 28.8 to 86.3%. Formulation IV, containing 10% butoxy polypropylene glycol and 4% methoxychlor was used by two herds, Nos. 8 and 9. Herd No. 8 was located in a 7-acre pasture with approximately 400 trees. Horn fly control in this herd ranged

Table 3.—Mean number of flies per animal per day and per cent control of horn flies and stable flies on four herds using cable-type back rubbers treated with formulation I, containing 50% butoxy polypropylene glycol and 5% methoxychlor. University Park, Pennsylvania. 1955.

Herd No.	Location of Back Rubber	Observation Period (Days)	Mean No. Horn Flies per Animal		Per Cent Control	Mean No. Stable Flies per Animal		Per Cent Control
			Treated	Check		Treated	Check	
1, 2, 3[a]	Pasture A (2 acres with 6 trees)	1– 5	0.2	155.3	99.9	1.6	14.0	88.6
		6–10	1.0	121.7	99.2	2.5	9.7	74.2
		11–15	0.8	105.7	99.2	4.5	12.7	64.6
1, 3		16–20	2.5	100.0	97.5	8.5	18.0	52.8
		21–25	0.9	130.0	99.3	8.7	8.0	0.0
4[b]	Pasture D (10 acres with 130 trees and 40 dead trunks and branches)	1– 5	23.0	35.0	34.3	7.5	14.0	46.4
		6–10	24.2	77.0	68.6	3.0	4.4	31.8
		11–15	15.0	125.0	88.0	2.8	2.3	0.0
		16–20	15.0	143.3	89.5	12.5	8.0	0.0
		21–25	34.5	185.0	81.4	12.5	9.5	0.0

[a] Herds No. 1, 2 and 3 were rotated twice during 8 weeks of observations so that each herd served as treated, check, and post-treatment animals for residual effect studies. The results obtained from the three herds in turn in each pasture were combined and averaged.
[b] Herd No. 5 in a nearby pasture served as check animals for herd No. 4.

Table 4.—Mean number of flies per animal per day and per cent control of horn flies and stable flies on four herds using cable-type back rubbers treated with four insecticide-repellent formulations. University Park, Pennsylvania. 1956.

Formulations	Herd No.	Location of Back Rubber	Observation Period (Days)	Mean No. of Horn Flies per Animal		Per Cent Control	Mean No. of Stable Flies per Animal		Per Cent Control
				Treated	Check		Treated	Check	
I[a]	6	Indoors (Barn)	1– 5	2.2	44.0	95.0	4.0	8.7	54.0
			6–10	1.4	18.1	92.3	0.8	6.6	87.9
			11–15	1.0	30.0	96.7	1.8	7.1	74.6
			16–20	1.6	26.7	94.0	3.8	10.4	63.5
			21–25	1.7	32.4	94.8	3.6	8.9	59.6
II[b]	6	Indoors (Barn)	1– 5	2.8	50.0	94.4	0.1	0.9	88.9
			6–10	4.8	31.0	84.5	0.4	3.2	87.5
			11–15	3.6	42.6	91.5	0.6	2.2	72.7
			16–20	2.3	23.0	90.0	1.7	2.5	32.0
			21–25	1.7	31.2	94.6	1.2	4.3	72.1
III[c]	7	Pasture A (2 acres with 6 trees)	1– 5	1.1	72.0	98.5	0.3	1.6	81.2
			6–10	2.7	63.7	95.8	0.6	4.1	85.4
			11–15	1.6	80.4	98.0	2.5	5.2	51.9
			16–20	5.6	54.6	89.7	4.6	7.2	36.1
			21–25	—	—	—	—	—	—
			26–30	1.6	88.5	98.2	1.2	5.9	79.7
			31–35	1.3	69.8	98.1	2.0	4.3	53.5
			36–40	1.0	47.7	97.9	1.0	3.8	73.7
			41–45	1.6	77.3	97.9	5.0	14.6	65.8
			46–50	5.7	91.3	93.8	4.2	5.9	28.8
			51–55	1.0	83.6	98.8	3.3	8.2	59.8
			56–60	1.0	72.8	98.6	1.0	7.3	86.3
			61–65	3.3	65.2	94.9	4.5	6.4	29.7
IV[d]	8	Pasture E (7 acres with 400 trees)	1– 5	3.0	93.8	96.8	2.0	5.9	66.1
			6–10	3.4	65.9	94.8	3.6	4.7	23.4
			11–15	1.2	41.3	97.1	1.5	1.7	11.8
			16–20	4.4	77.3	94.3	4.6	14.6	68.5
			21–25	3.4	82.2	95.9	5.8	7.3	20.5
			26–30	0.9	101.8	99.1	0.2	9.4	97.9
			31–35	3.7	66.7	94.4	3.0	5.4	44.4
IV	9	Pasture F (40 acres with 40 trees)	1– 5	3.6	91.5	96.1	4.2	7.3	42.5
			6–10	3.5	106.2	96.7	6.6	5.7	0.0
			11–15	7.0	75.2	90.7	10.3	12.4	16.9
			16–20	—	—	—	—	—	—
			21–25	2.2	18.8	88.3	8.9	10.0	11.0
			26–30	2.9	40.6	92.8	9.0	11.4	21.0
			31–35	2.8	16.9	83.4	7.9	11.1	28.8

[a] Containing 50% butoxy polypropylene glycol and 5% methoxychlor.
[b] Containing 8.6% butoxy polypropylene glycol, 1% methoxychlor, 0.6% Lethane 384, and 0.03% synergized pyrethrins.
[c] Containing 5% methoxychlor.
[d] Containing 10% butoxy polypropylene glycol and 4% methoxychlor.

from 94.3 to 99.1%, while stable fly control varied from 11.8 to 97.9% during the entire observation period of 32 days. Herd No. 9 was located in a 40-acre pasture with 40 trees. Observations, made over a period of 34 days, showed that horn fly control varied from 83.4 to 96.7%, while stable fly control from 0 to 42.5% (fig. 4).

The per cent control of the horn fly and stable fly reported above is an average of the results for every 5-day period. In order to compare the overall effectiveness of the formulations tested, the mean per cent control for the first 25 days of observation was determined for each formulation, except formulation III for which the first 30 days were used. The mean per cent control of the horn fly was 94.1 with formulation I, 92.0 with formulation II, 96.2 with formulation III, 94.4 with formulation IV used by herd No. 8, and 94.5 with formulation IV used by herd No. 9. The standard deviations from the mean of per cent control varied from 2.3 to 4.8. The mean per cent control of the stable fly was 68.1 with formulation I, 76.6 with formulation II, 74.9 with formulation III, 42.1 with formulation IV used by herd No. 8, and 16.8 with formulation IV used by herd No. 9. The standard deviations from the mean of per cent control varied from 15.5 to 30.0. The comparative effectiveness, during a 25-day period, of the four formulations is graphically shown in figure 5.

An analysis of the data for horn fly control established that the differences between fly populations on treated and check animals were highly significant but that the differences in the degrees of control among the formulations tested were not. Evidently, as far as the control of the horn fly was concerned, there was no significant difference in the efficiency of the rubbing device whether it was installed indoors or outdoors, in a 2-acre or 40-acre pasture, among a few trees or at the border of a heavily wooded area. It must be emphasized, however, that all

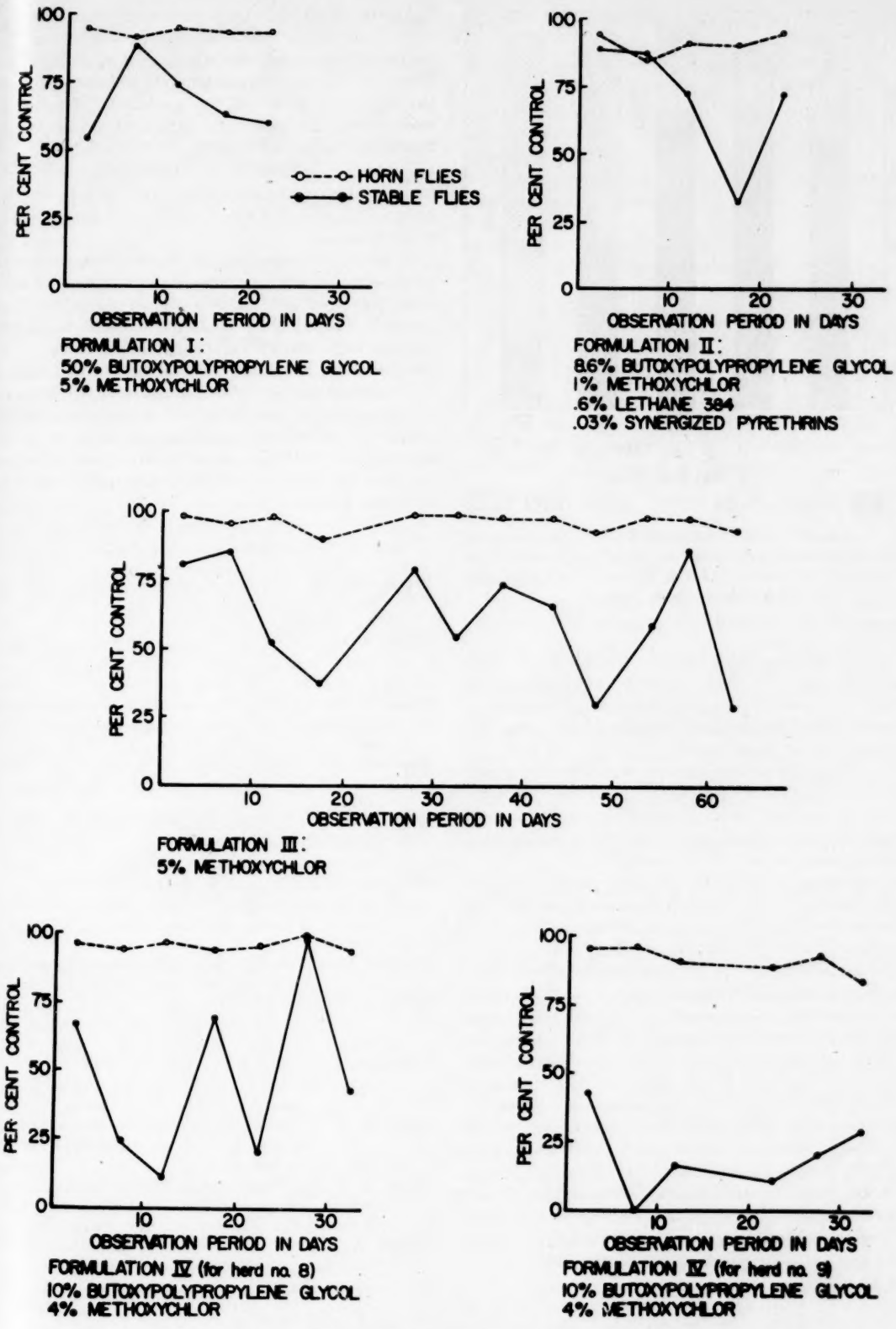

FIG. 4.—Effectiveness of four insecticide-repellent formulations tested on the cable-type back rubber for the control of biting flies on cattle. University Park, Pennsylvania. 1956.

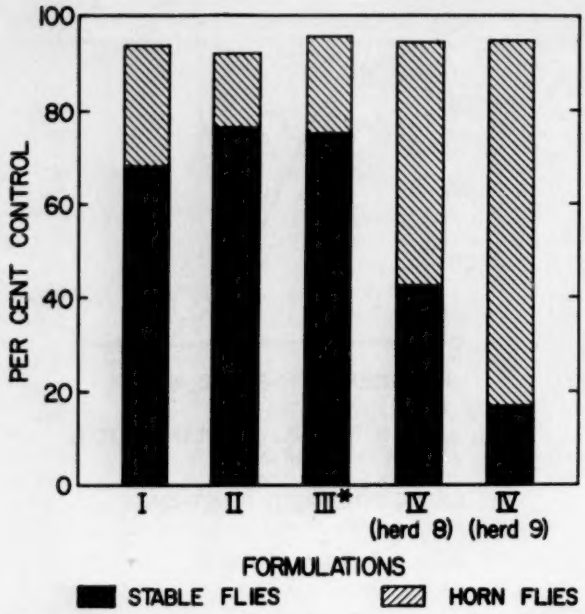

FIG. 5.—Comparative effectiveness, during a 25-day period, of four insecticide-repellent formulations tested on the cable-type back rubber for the control of biting flies on cattle. University Park, Pennsylvania. 1956.

* Thirty-day period.

back rubbers used in the tests were so located as to make them readily accessible to the animals. It should be noted here that tests made in 1955 indicated that the presence of numerous dead trunks and branches might reduce the effectiveness of the back rubber.

The fact that the rubbing device was effective indoors as well as outdoors made it desirable from the standpoint of saving material and labor. It could be installed inside a basement barn or a straw shed of good size and be sheltered from heavy rains and hot summer sun.

The consistently excellent protection provided by 5% methoxychlor in diesel oil against the horn fly should be of practical interest to many farmers. Tests made in the 2 years indicated that the addition of butoxy polypropylene glycol to methoxychlor did not enhance the effectiveness of the latter to any significant degree.

The results of the tests conducted during the summer of 1956 also confirmed the findings of the preceding year that while the back rubber provided consistently excellent control of the horn fly, it was, nevertheless, unsatisfactory against the stable fly. An examination of table 4 clearly shows that none of the four formulations accomplished effective control of this insect. Also, as shown in figure 4, there was a great deal of variation and fluctuation in the data relative to the effectiveness of each formulation against the stable fly. Because of this variation, it seems premature to draw any conclusions regarding the comparative effectiveness of the materials for stable fly control.

The fact that the cattle were rarely seen rubbing their legs on the device might account for the less satisfactory control of the stable flies which feed mainly on the legs. Moreover, it was commonplace to observe cattle wading through mud holes in the pastures. This, no doubt, washed away any material that might have protected the legs from the stable flies. There was also the possibility that part of the stable fly population had developed resistance to the materials used. These factors might have been responsible for the variable and unsatisfactory results obtained.

None of the animals used in the back rubber experiments showed any apparent adverse effects. Injury, however, was recorded for two month-old calves that had access to a back rubber treated with formulation IV, containing 10% butoxy polypropylene glycol and 4% methoxychlor in diesel oil. The rubbing unit in this case was not included in the experiments, hence, no accurate observations were recorded. The animals became listless, refused to eat and lost large patches of hair from areas of their bodies. After they were denied access to the rubbing unit, the calves recovered and eventually regained all of their lost hair.

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Susceptibility of Life Stages of *Sitophilus oryza* to Various Fumigants[1]

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ABSTRACT

Results are given of the susceptibility of the egg, larval, pupal, and adult stages of the rice weevil, *Sitophilus oryza* (L.), in infested wheat fumigated with acrylonitrile, carbon bisulfide, chloropicrin, ethylene dibromide, ethylene dichloride, and methyl bromide.

If the egg stage is omitted, the order of decreasing susceptibility to all fumigants except ethylene dichloride is larva, adult and pupa. In comparison with the other stages, the eggs are resistant to carbon bisulfide and relatively resistant to methyl bromide.

In the fumigation of infested stored products, the stage of development of the insect may be as important as the species involved. There may be a greater difference in susceptibility to a fumigant between two stages in the development of one insect species than between similar stages of two separate species. In recommending a fumigant for use it is important to know whether the fumigant will kill all the life stages of the insects involved. Considerable work has been done comparing the toxicity of fumigants to a particular stage of different insects, but, in general, the data are limited as to the effect of various fumigants on all stages of development of a particular insect species. Sun (1947), in a comprehensive study, determined the susceptibility of the various stages and of the ages within a stage of *Tribolium confusum* Duv. to carbon bisulfide. The main object of the present investigation has been to determine the susceptibility of the egg, larval, pupal, and adult stages of the rice weevil, *Sitophilus oryza* (L.), to several fumigants.

MATERIALS AND METHODS.—In order to obtain eggs, larvae, and pupae for the fumigation experiments, soft western wheat of 14% moisture content was exposed to large numbers of adult rice weevils for 3-day periods. The adults were then removed and the infested wheat was kept at 80°F. and 60% relative humidity. In this manner, eggs, larvae, and pupae of plus or minus 1½ days in age were made available. The presence of pupae was determined by cutting and examining infested kernels. When a minimum of 90% of the kernels contained pupae, they were fumigated. Because of unevenness in development it was difficult to get 100% pupal-infested kernels. This variation in development was such that an occasional adult as well as a few larvae could be found in the same culture with many pupae. Owing to this overlapping the ages of the pupae were unknown.

The eggs used in these experiments were plus or minus 1½ days old. The larvae were half-grown (midway between eggs and pupae in age). Adults ranged in age from 2 to 4 weeks. In each test 50 to 60 adults were used in plastic screen cages without food.

Prior to the withdrawal of the aliquots of infested wheat for fumigation, the cultures being used for the egg, larval, and pupal comparative fumigations were thoroughly mixed to ensure a random sample. Aliquots of 100 grams of infested wheat were used for each test, and similar aliquots were set aside for controls as indices of infestation.

Fumigations were conducted in 28-liter air-tight chambers with built-in agitators operated from the outside by a motor. The four stages of the weevil, in separate cages, were placed in a fumigation chamber. The fumigants were applied by a microburette, and exposures were for 2 hours at a temperature of 80° F.

Adult mortality was determined in 5 days. The aliquots of infested wheat were placed in half-pint jars sealed with a cloth cover and held at 80° F. and 60% relative humidity until all adults had emerged. These adults were then sifted out of the wheat and counted. Mortality was based on the number of adults emerging

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Table 1.—Dosages of various fumigants required for 50% kill (LD-50) and 95% kill (LD-95) of life stages of *Sitophilus oryza* at exposures of 2 hours (temperature, 80° F.).

FUMIGANT	PER CENT KILL	LETHAL DOSAGE (mg./l.) REQUIRED FOR: Eggs	Larvae	Pupae	Adults	LR TO R[a]
Acrylonitrile	50	1.0	1.0	2.9	2.2	E=L<A<P
	95	1.9	1.9	5.7	3.3	E=L<A<P
Carbon bisulfide	50	190.0	48.0	95.0	55.0	L<A<P<E
	95	250.0	70.0	207.0	87.0	L<A<P<E
Chloropicrin	50	7.3	5.6	6.9	7.4	L<P<E=A
	95	19.0	14.2	23.5	20.5	L<E=A<P
Ethylene dibromide	50	0.7	1.7	5.3	3.3	E<L<A<P
	95	1.8	4.4	10.1	7.1	E<L<A<P
Ethylene dichloride	50	197.0	197.0	>270.0	177.0	A<E=L<P
	95	>270.0	>270.0	>270.0	>270.0	
Hydrocyanic acid	50	1.1	3.2	6.4	7.1	E<L<P<A
	95	4.8	6.0	37.0	31.0	E<L<A<P
Methyl bromide	50	10.7	5.8	14.5	7.5	L<A<E<P
	95	18.5	10.1	22.2	13.5	L<A<E<P

[a] Least resistant to resistant.

from the control aliquots, minus the number emerging from the treated, divided by the number in the control, times 100 to give the percentage of kill.

Fumigants used were acrylonitrile, carbon bisulfide, chloropicrin, ethylene dibromide, ethylene dichloride, hydrocyanic acid, and methyl bromide. To arrive at a dosage-mortality curve, five dosages of ethylene dichloride and 10 or more dosages of the remaining fumigants were used, each dosage being replicated three times. The LD-50 and LD-95 were determined from probit log-concentration curves.

RESULTS.—If in comparing the LD-95 of the fumigants the egg stage is omitted, it will be noticed in table 1 that with every fumigant except ethylene dichloride, for which no comparison can be made, the decreasing order of susceptibility to the fumigants is larva, adult, and pupa. The pupa, probably having the lowest rate of metabolism of these three stages, was the most resistant. The egg stage appeared to be relatively susceptible to acrylonitrile, ethylene dibromide, and hydrocyanic acid; resistant to carbon bisulfide, and relatively resistant to methyl bromide.

The ratios of fumigant dosages required to kill 95% of the least resistant and the most resistant stages were 1:3 for acrylonitrile, 1:3.6 for carbon bisulfide, 1:1.7 for chloropicrin, 1:5.6 for ethylene dibromide, 1:7.7 for hydrocyanic acid, and 1:2.2 for methyl bromide. Published toxicity data show that in some cases these ratios between stages of rice weevil are greater than ratios between adults of various other stored-product insects.

In comparing the relative effectiveness of the fumigants at LD-95 it was found that acrylonitrile and ethylene dibromide were the most toxic of those tested to all stages of the rice weevil, followed by hydrocyanic acid, methyl bromide, and chloropicrin; carbon bisulfide and ethylene dichloride being the least toxic.

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Malathion for Control of Chicken Mites on Hens in Wire Cages[1]

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ABSTRACT

Aqueous sprays containing 0.5% and 1.0% actual malathion by volume, respectively, were applied to community-type cages with wooden frame work by means of a power-driven sprayer for the control of the chicken mite, *Dermanyssus gallinae* (De G.). Both concentrations of malathion provided good control of the chicken mite and, in this instance, of the northern fowl mite, *Ornithonyssus sylviarum* (C. & F.).

Complete control of chicken mites present in individual-type cages constructed entirely of wire was obtained with a single application of 4% malathion dust.

The chicken or roost mite, *Dermanyssus gallinae* (DeG.), may attack poultry throughout the year in southern California. In this area, the wire mesh cage (Dougherty & Belton 1929) commonly built with a wooden supporting framework is a type of hen house widely used on commercial poultry ranches (figures 1 and 2). The open mesh floor of the cage is raised off the ground, and litter is not used. The cages vary in size from the individual type (for 1 or 2 hens) to the community type (for as many as 90 hens per cage).

Although control of the chicken mite has not been difficult, in general, and several established control measures have been reasonably satisfactory, pesticides better suited to modern poultry operations have been needed. A number of recently developed organic insecticides have been tested for control of the chicken mite by applying the material to the roosts and structural portions of hen houses having a ground-level floor. Smith (1952) reported results obtained in Texas with several chlorinated hydrocarbon insecticides used in spray or dust. Moore & Schwardt (1954), in New York, obtained very satisfactory control with 1% malathion in spray as one of several experimental treatments tested against the chicken mite. Results reported by Raun (1956) demonstrated that malathion at a concentration of either 1% in spray or 4% in dust gave effective control of the chicken mite in Iowa. Furman & Weinmann (1956) observed the complete disappearance of chicken mites in a hen house treated with 3% malathion spray.

The extensive use of wire cages in commercial poultry operations in southern California indicated the desirability of experiments to investigate procedures and types of insecticide formulations suitable for application to such enclosures. Conditions apt for this purpose existed at two ranches infested with the chicken mite, one near Anaheim and the other near Colton. Malathion was the pesticide of choice, and the high efficiency found by previous workers offered good reason for trying a lower dosage.

MATERIALS AND METHODS.—At Anaheim, the hens were housed in community-type cages having wire mesh floors, wooden framework, and wooden nest boxes. Each cage contained 35 to 40 hens. The wooden parts of the cages were infested with the chicken mite, and concurrently a relatively light infestation of the northern fowl mite, *Ornithonyssus sylviarum* (C. & F.), was found on the hens. Six cages infested with both species of mites were selected for treatment, and several adjoining cages were used for untreated check counts.

Estimates of the chicken mite infestation were made by counting the mites at each of six sites on each pen at

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The pesticide chemicals discussed may not be used unless a tolerance has been established or an exemption from the requirement of a tolerance has been granted for each specific use, irrespective of the information contained in this report.

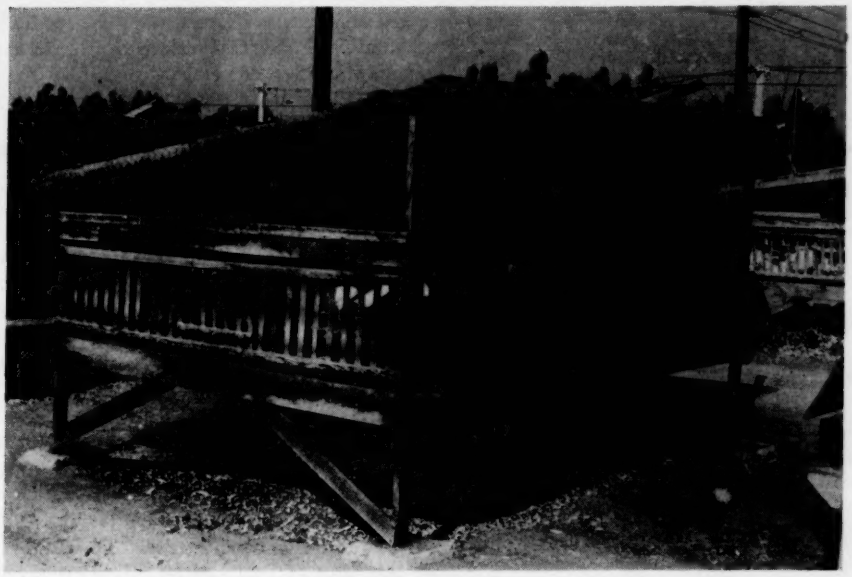

FIG. 1.—A community-type wire mesh cage with wooden framework used by a commercial poultry ranch to house hens.

each count interval. At each site the debris that could be dislodged readily from cracks or crevices at the ends of slats, at framework corners, etc., was pushed or scraped out of an area of approximately 1 square inch and collected in a small shallow pan. The mites that could be seen quickly and easily with the unaided eye were counted, and the sample of debris was discarded.

The infestation of northern fowl mite was expressed in terms of the percentage of hens infested. Ten hens were caught at random and examined in each cage at each count interval.

FIG. 2.—Arrangement of a large number of community-type wire mesh cages in use at a commercial poultry ranch to house hens.

Malathion was applied in aqueous spray at each of two concentrations, 0.5% and 1.0% actual malathion by volume. The mixtures were prepared with a proprietary 57% emulsifiable concentrate formulation. Each treatment was replicated three times. Spray was applied by means of a power-driven pump operated at 150 p.s.i. and was directed only to the inner and outer surfaces of the wooden structural portions of the cages. The hens were not taken out of the pens during treatment but were herded out of the way of the spray stream, and every precaution was taken to keep the direct spray off the hens. Some of the spray material reached the hens, unintentionally, however, from drift and from brushing against the wet surfaces of the cages before the spray dried. Before spraying, the eggs were collected and the feed and water containers were removed.

At Colton, the individual- or compartment-type of wire cage was used, and wire was the sole material used in the structure. Eight single cages were joined together to form a block unit. Each compartment of the block could accommodate two hens. While chickens in this type of cage are just as likely to be infested with the northern fowl mite as those in other types of poultry houses, an infestation of the chicken mite is rare, since the points of contact between the various pieces of wire do not usually leave satisfactory places for the mites to hide during the day. At Colton, dust and spilled feed had accumulated on the floors of the compartments and on the rungs, corners, and edges at the front and back. Chicken mites were hiding within the layer of accumulated dust and feed and in webbing which had formed across the heavy-gauge-wire loops used to fasten adjoining compartments together.

Counts of the chicken mites were made in the same way at Colton as at Anaheim. Samples were taken at each of six sites in each block of eight compartments.

Two rows of five blocks of cages each were used in the trial, one row treated and the other untreated. A con-

Table 1.—Reduction in populations of chicken mite and northern fowl mite at various intervals after application of malathion as indicated to cages housing hens for egg production.

Days After Treatment	No. of Chicken Mites per Sample				Per Cent Hens Infested With Northern Fowl Mite		
	Untreated	Per Cent Malathion Applied 0.5	1.0	4.0	Untreated	Per Cent Malathion Applied 0.5	1.0
Aqueous Spray in Community-Type Cages							
0[a]	59	63	94		30	40	76
1	71	0.1	0		20	0	1
7	60	0	0		10	0	0
14	39	0	0		20	0	0
21	45	0	0		30	0	0
28	40	0	0		30	0	0
64	27[b]	0	0		20[b]	0	0
Dust in Individual-Type Cages							
0[a]	37		60	60			
7	30		0	0			
14	21		0	0			
21	23		0	0			
28	27[b]		0	0			

[a] Pretreatment.
[b] At the grower's request, cages left untreated for check counts were treated with malathion.

ventional hand-operated, bellows-type duster was used to blow 4% malathion dust across the floor of each compartment. The outlet orifice of the duster was held in front of the compartment at floor level. A visible film of the pesticide was left on the surfaces of the accumulated material harboring the mites. The hens were not taken out of the cages, but only the feet and shanks of the legs were hit by the malathion dust. The eggs were collected, and troughs for feed and water were removed before the application.

DISCUSSION OF RESULTS.—Counts of mites at the two experimental sites were made each week during the first month after treatment. After 1 month at Colton and 2 months at Anaheim the poultry growers requested treatment of the untreated cages left for check counts. The data for the first 2 months are presented in table 1. Observations were continued and counts were made each month for a total period of 7 months after the initial treatment, comprising the seasons of summer, fall, and winter. No mites were found in any of the treated pens during this time. The count made in the untreated check cages offer satisfactory evidence that the disappearance of mites in the treated cages was due entirely to the malathion applied.

Since control of the chicken mite in community cages at Anaheim was as effective with 0.5% malathion spray as with 1.0% spray, it is possible that a concentration of malathion lower than 0.5% might be used when a power-driven sprayer is employed.

Both concentrations of malathion controlled the northern fowl mite in the community cages at Anaheim, but the initial percentage of hens infested with this pest was not particularly high and this may have been a contributing factor in the control attained. The treatment was directed against the chicken mite, and the spray was applied to the surfaces of the cage although some of the spray reached the hens unintentionally by drift. Whether the same concentrations of malathion and type of spray application would be as effective consistently for control of the northern fowl mite is open to question.

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Insecticidal Seed Treatments for Cotton[1]

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ABSTRACT

Replicated tests conducted during three growing seasons (1954, 1955, 1956) at College Station, Texas, have shown that several systemic insecticides (demeton, Am. Cyanamid 12008 and 12009, Thimet and Bayer, 19639) applied as seed treatments adequately protected young cotton plants from thrips for a period of 4 to 6 weeks after the planting date. These materials varied in phytotoxicity which was manifested by reduced emergence or somewhat delayed fruiting under unfavorable conditions for plant emergence and growth. No significant reduction in yield resulted from these phytotoxic effects.

Tests have been conducted at the Texas Station for three seasons (1954, 1955, 1956) to evaluate the usefulness of insecticidal seed treatments for the control of early season cotton pests. This type of application has several advantages. A single seed treatment may replace several foliage applications, the plant is protected from the time of emergence when it is especially vulnerable, and foliage applications can be dispensed with at a time when spraying and dusting are often difficult because of unfavorable weather conditions. Since both the phytotoxic effects and the length of the period of protection from this type of treatment may differ with varying environmental conditions, trials in different seasons and in different localities are necessary to obtain an adequate estimate of the value of seed treatments.

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Table 1.—Results of cotton seed treatments, Experiment 1, College Station, Texas. 1954.

TREATMENT (1 LB./BU.)	PLANTS ON 50 FT. OF ROW		WEIGHT OF 50 PLANTS (GRAMS)		THRIPS ON 100 LEAVES[a]	YIELD PER ACRE (POUNDS)		
	May 5	May 19	May 5	May 19		Sept. 14	Oct. 2	Total
Demeton	336	217	17.1	33.5	4.2	576	964	1540
Am. Cyanamid 12008	323	259	16.9	34.0	4.5	546	934	1480
Am. Cyanamid 12009	323	229	17.4	30.2	4.3	556	954	1510
Untreated	528	467	21.9	29.5	11.5	566	980	1546
L.S.D. at 5% level	54	47	3.3	ns	3.0	ns	ns	ns

[a] Seasonal average.

In the tests described herein, insecticides were applied to mechanically delinted seed as dust formulations during the week preceding planting except for one treatment 1956 which was a commercial application on acid-delinted seed. All mechanically delinted seed was planted with tractor drawn, four-row planters adjusted to deliver 32 pounds of seed per acre. Acid-delinted seed was planted by a similar planter at the rate of 25 pounds per acre. In this series of experiments the only insect populations that were large enough and sufficiently consistent to furnish reliable estimates of insecticidal effectiveness were thrips, principally *Frankliniella fusca* (Hinds) and *F. tritici* (Fitch).

EXPERIMENT 1.—Results are shown in table 1. This test had a randomized block plot arrangement with four treatments and six replicates. Plots were 4 rows wide and 1/10-acre in size. Cotton was planted April 24 and emerged to a stand by May 5. Temperatures during this period were not unfavorable for germination, but 6 of these days were rainy with a total precipitation of 3.93 inches. Eleven days after planting, the plots treated with carbon formulations of demeton, Am. Cyanamid 12008 and 12009 had approximately 60% as many plants as the untreated plots, and the average weight of the plants was about 77% of the weight of plants taken from the untreated plots. Two weeks later there were approximately half as many plants on the treated as on the untreated plots, but the plant weights were no longer significantly different. The number of plants remaining on the treated plots were sufficient for an adequate stand. Thrips control was excellent until 4 weeks after planting, and fair control was maintained for another 2 weeks. The narrow 4-row plots may have limited the increase of thrips populations on the untreated plots. There were no significant differences in earliness of maturity nor total yield due to the treatments.

EXPERIMENT 2.—This experiment was conducted to test the effectiveness of demeton, Am. Cyanamid 12008 and two dosages of Thimet as seed treatments for thrips control (table 2). A spray treatment consisting of three early-season applications of toxaphene to the young plants and a check that had carbon only applied to the seed were included for comparison. The check with carbon was included because the other seed treatments applied were carbon formulations. A randomized block plot arrangement was used with four replications of each treatment. This experiment was planted April 21 when very good soil sonditions and temperatures for germination existed and the seedlings emerged to a stand by April 25.

Early stand counts indicated some inhibition of early growth by all seed treatments. It was pronounced with demeton but relatively slight with Thimet and Am. Cyanamid 12008. Final stands on all plots were adequate

Table 2.—Results of cotton seed treatments, Experiment 2, College Station, Texas. 1955.

CRITERIA AND DATES	TREATMENTS						L.S.D. AT 5% LEVEL
	Thimet		12008	Demeton	Carbon		
	0.5 lb./A.	1 lb./A.	1 lb./A.	(1 lb./A.)	(2 lb./A.)	Toxaphene Spray	
Plants per 10 ft of row							
April 25	34.5	33.1	26.4	10.1	44.0	46.8	20.8
April 28	53.8	55.8	47.9	32.6	64.6	74.2	32.3
May 3	59.9	61.6	54.8	42.6	68.3	77.5	33.9
May 7	68.5	73.3	66.0	57.8	77.6	92.8	23.7
Thrips per 100 leaves (Average for May)	3.8	2.4	4.3	6.9	33.8	21.8	6.9
Squares per 100 ft. of row June 9	772	677	650	574	660	788	140
Blooms on 1/10 acre (Average June 24, 27, and July 1)	812	792	713	553	751	810	114
Bolls per 100 ft. of row July 8	637	569	500	406	453	523	145
Pounds seed cotton per acre Aug. 17 and Sept. 15	1336	1418	1348	1542	1536	1376	ns

Table 3.—Results of cotton seed treatments, Experiment 3, College Station, Texas. 1956.

Criteria	Thimet	Carbon	Acid Delinted		Bayer 19639		Aldrin	Granulated Heptachlor		Toxaphene Sprays	L.S.D.
			Thimet	No Thimet							
Active ingredient (lb./a.)	0.92	1.56	1.06	—	0.93	1.88	0.47	0.49	1.07	3.80[a]	
Seed/Acre (lb./a.)	29.4	25.0	26.5	26.7	29.8	30.1	30.3	31.2	32.0	32.0	
Thrips on 100 leaves											
May 3	0.0	11.0	0.0	17.5	0.5	0.0	4.5	5.8	4.3	10.3	10.2
May 14	0.0	10.3	0.0	11.0	0.8	0.0	4.0	4.3	5.0	2.3	3.0
May 18	1.0	8.5	1.0	8.8	1.5	0.0	3.0	5.0	1.8	0.8	6.9
May 25	9.0	19.7	8.3	24.0	10.5	9.3	18.7	15.3	13.3	3.0	7.6
June 4	30.0	38.0	27.3	47.0	18.8	26.3	35.8	26.5	22.8	10.5	16.2
Seasonal average	8.0	17.5	7.3	21.7	6.4	7.1	13.2	11.4	9.4	5.4	4.3
Plants/acre[b]											
May 3	74,945	91,803	65,013	69,783	100,820	81,675	68,084	74,814	85,590	93,567	15,593
May 18	55,474	65,536	40,707	57,957	76,121	59,590	51,945	60,113	70,044	59,067	10,860
Squares/100 ft.											
June 8	171	189	165	196	223	201	184	200	165	218	49
June 13	364	414	326	360	420	376	362	380	380	470	89
June 18	631	650	539	550	598	596	584	538	581	728	85
Average	389	418	344	369	414	391	377	373	375	472	55
Bolls/100 ft.											
Aug. 8	556	579	531	605	568	551	537	591	538	648	NSD
Open bolls/100 ft.											
Aug. 8	345	408	336	384	381	374	378	398	360	477	74
Seed cotton/acre	743	732	629	626	694	716	722	737	708	796	ns

[a] Total for 3 applications 5-10-56, 5-18-56 and 5-29-56.
[b] Corrected for amount of seed planted.

for maximum yields. Excellent thrips control resulted from the use of all the systemic seed treatments and it was better than that obtained with three early-season applications of toxaphene to the foliage. Control of thrips began to decrease in the demeton-treated plots during the fifth week after planting and in the plots treated with other systemics during the sixth week. Fruiting was delayed somewhat by the demeton treatment, but any difference in earliness of harvesting due to this delay was obscured by variations in the soil and by drouth. There were no significant differences in yields due to treatments.

EXPERIMENT 3.—This test was designed to compare a number of insecticidal treatments, both systemic and non-systemic (see table 3). Two dosages of Bayer 19639 were tried, and two types of applications of Thimet were tested, a dust on reginned seed and a solid coating on acid-delinted seed. A seed treatment with carbon alone, a furrow treatment with granulated heptachlor and a foliar treatment of three early-season applications of toxaphene spray were included for comparisons. A randomized block plot arrangement was used. Soil moisture was deficient at planting time and unseasonably low temperatures prevailed. Plants from seed planted on April 18 required almost 2 weeks to emerge.

There were more plants May 3 on plots where the seed was untreated (toxaphene sprays), treated with carbon only or treated with 1 pound of Bayer 19639 per bushel than on the plots where the seed was treated with Thimet, aldrin or heptachlor, or on those where acid-delinted seed without insecticidal treatment was planted. Final stands on all plots were sufficient for maximum yields in a dry year. Thrips control on seedling cotton was best on the plots planted with treated seed. Later, there were fewer thrips on the sprayed plots. The furrow treatments and seed treatments with chlorinated hydrocarbons caused some reduction in thrips. Plants on all plots planted with treated seed were slower fruiting and slower to mature than those on plots which received foliage sprays. There were no significant differences in yields.

EXPERIMENT 4.—This test was a randomized block, split-plot experiment designed to compare seed treat-

Table 4.—Effects of different early season treatments on thrips infestation, fruiting and yield, Experiment 4, College Station, Texas. 1956.[a]

Criteria	Treatments		
	Three Toxaphene Sprays	Thimet Seed Treatment	Thimet Seed Treatment+ One Toxaphene Spray
Thrips on 100 leaves			
May 2	3.0	0.1	0.0
May 13	1.3	0.5	0.3
May 18	2.0	1.4	2.0
May 25	3.7	9.3	8.8
June 4	8.5	27.5	25.3
June 14	20.5	24.3	17.0
Seasonal average	6.5	10.5	8.9
Squares per 100 ft.			
June 7	219	185	174
June 12	433	369	337
June 18	810	707	666
Average	487	420	392
Bolls on 100 ft.			
Aug. 8	601	487	486
Open bolls on 100 ft.			
Aug. 8	444	343	336
Seed cotton per acre	625	498	517

[a] Each entry an average of 12 plots.

ments, foliar sprays, and a combination of both methods for early-season cotton insect control in combinations with three different late-season control schedules (table 4). Thimet was applied at the rate of 1 pound per bushel of seed and 1 bushel of seed per acre was planted. Toxaphene at an average rate of 1.27 pounds per acre was used for the three applications to the foliage. Planting conditions, date of planting and emergence were the same as those in Experiment 3.

Early sprays were somewhat more effective than seed treatments for thrips control. This was due principally to better control in late May and early June. Control with seed treatments had definitely declined by May 25, five weeks after planting. Fruiting and maturation of the crop were slower on the plots planted with treated seed, but yields were not significantly different. There were no important interactions between the early-season and late-season control programs.

DISCUSSION.—In the 3 years that applications of systemic insecticides to cotton seed have been tested at College Station, quite different planting conditions have prevailed. During the 11-day period from planting to emergence in 1954 the mean daily temperatures averaged 72° F., but there was rain on 6 days with a total precipitation of 3.93 inches. The phytotoxic effect in 1954 was manifested principally by poor emergence. The 1955 season afforded good planting conditions with an average daily mean temperature of 76° F. with adequate soil moisture and no rain. Only 5 days were required for emergence. Under these conditions only demeton showed any appreciable phytotoxicity. In 1956 soil moisture at planting time was critically low, and the 2-week emergence period had an average daily mean temperature of only 66° F. with the lowest daily minimum 40° F. The most important phytotoxic effect in 1956 seemed to be slight delay in fruiting and maturity.

The period of protection from thrips afforded by seed treatments with systemic insecticides can be more accurately estimated from planting time than from emergence time of the seedling cotton. Based on the data reported herein, 4 to 5 weeks of almost complete control from the date of planting may be expected plus another 2 weeks of variable effectiveness. The time required for emergence reduced the period of plant protection.

Recent Attempts to Establish Sugarcane Borer Parasites in Louisiana[1,2]

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ABSTRACT

Tests are being conducted to determine the possibilities of obtaining economic control of the sugarcane borer (*Diatraea saccharalis* (F.)), a pest of foreign origin, with larval parasites. Two tachinids, the Amazon fly (*Metagonistylum minense* Towns.) and the Cuban fly (*Lixophaga diatraeae* (Towns.)), are imported from Trinidad, B.W.I. and released in Louisiana sugarcane fields. In the 1955–56 seasons 55,781 parasites were released on 34 plantations. One hundred and seventy-five release areas were surveyed for establishment during the fall surveys. There were no recoveries of the Amazon fly. The Cuban fly was recovered on all plantations with an average parasitization of 26%. It became established in fields up to 2 miles from the nearest release points. This species has survived the winter in summer plant sugarcane which is not harvested in the year of planting. The best results were obtained in fields in which the parasite overwintered. Attempts to establish from India four species of parasites of stalk borers and the pink bollworm have been unsuccessful. A borer parasite introduced from Florida in 1944 was recovered in 1956.

Of all the insects that attack sugarcane in the continental United States, the sugarcane borer (*Diatraea saccharalis* (F.)), a pest of foreign origin, is the most destructive. In Louisiana alone it causes an estimated annual loss of about 5 million dollars. A number of attempts have been made at the Houma, La., Laboratory to obtain effective biological control of the borer. Thirteen species of parasites that attack this insect and related species in this country or in foreign countries have been introduced at various times since 1915, with different degrees of success. Two tachinids, the Amazon fly (*Metagonistylum minense* Towns.) and the Cuban fly (*Lixophaga diatraeae* (Towns.)), were the most effective and were recovered for several years, an indication of their ability to survive the field conditions and winters that prevail in Louisiana. However, neither of these parasites increased sufficiently to be of value in borer control.

Climatic conditions are not as favorable for the biological control of the borer in Louisiana, where the insect has a winter dormant period, as they are in Florida and other areas where all stages are present throughout the year and the parasites can breed continuously. However, present cultural practices and a cycle of relatively mild winters are considered more favorable for the establishment and possibly maintenance of larval parasites than they were during the periods 1915–20 and 1929–41, when most of the introductions were made.

It now appears likely that the Amazon and Cuban flies could become adapted to Louisiana conditions and be of economic value in the control of the borer. Also the successful introduction of some of its parasites in southern Florida and certain areas in the Caribbean, coupled with the increased costs of insecticide treatments, has stimu-

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[2] These studies were conducted in cooperation with the South Coast Corp., Southdown Sugars, Inc., Milliken and Farwell Inc., Valentine Sugars, Inc., Lafourche Sugars, Inc , Hughes Breaux, the Louisiana Agricultural Experiment Station, and the American Sugar Cane League of the U.S.A.

lated interest among growers for the use of parasites here. Consequently additional introductions have been made.

In 1953 releases were made, and the parasites became successfully established. This project was expanded during the next 3 years. Data on the 1953-54 releases and recoveries, a possible explanation of the success in establishment, and technique for handling the parasites have been published (Charpentier 1954, 1956). This paper presents data on the 1955 and 1956 introductions.

In 1955 from April 27 to October 2 a total of 25,748 parasites, reared from puparia purchased by the cooperating growers from J. A. Carmichael, Verdant Vale Estate, Arima, Trinidad, B.W.I., were released on 19 plantations at the rates of 5, 10 and 25 per acre, based on the severity of the borer infestation. In addition 101 Cuban flies were released in a rice field heavily infested with the sugarcane borer. Many of the parasites were released too late in the season for maximum effectiveness. From September 21 to November 2 ninety release areas were surveyed for establishment. There were no recoveries of the Amazon fly, although it had been recovered in a preliminary examination in August. Although the Amazon fly made up only 36% of the release number in 1954, the average percentage parasitization by it during that fall was 16 as compared with 13 for the Cuban fly. There were no recoveries of the Cuban fly in the rice field.

During the 1956 season 30,033 parasites were released on 15 plantations, and during the fall 85 representative release areas were surveyed. The Amazon fly apparently failed to become established. The Cuban fly, as in every other release year, was recovered on all the plantations. On a group of seven plantations south of Houma there was a 50% parasitization or higher in 18 of the 36 fields surveyed, with an average of 46% for the group. The 1955 parasitization for the same area was 34%. As in previous years, there were no significant differences in percentage of parasitization for the different release rates. The releases and recoveries for both years are summarized in table 1.

Surveys to determine spread have shown that the parasite became established in fields ½, 1, and 2 miles from the nearest release points.

The Cuban fly has demonstrated ability to survive the winter in fields of unshaved summer plant. Appreciable numbers have survived a minimum temperature of 23° F. in such fields. The best results in the 1954 season, a 75% parasitization, were obtained from an overwintering population on a plantation that had received 548 parasites in 1953 and none in 1954. In another field ¼ mile away there was a 46% parasitization from the same population. The highest parasitization of the 1955 season, 87%, was also recorded in a 1954 unshaved field of summer plant in which a 1954-55 overwintering population was augmented with some 1955 liberations. By December 1956 only on one of the many release plantations had the Cuban fly been able to maintain itself in cane trash left in the fields at harvest.

Table 1.—Releases and recoveries of parasites of the sugarcane borer in the Houma, La., area.

Year	Number of Parasites Released		Number of Borers and Parasites Collected	Per Cent Parasitization by	
	Amazon Fly	Cuban Fly		Amazon Fly	Cuban Fly
1955	1,043	24,705	3,203	0	19
1956	2,622	27,411	972	0	33
Total or average	3,665	52,116	4,175	0	26

In the 1955 harvest season parasitization and borer-infestation records were obtained on 100 stalks in each of 15 fields on eight plantations. The percentage of joints bored appeared to be higher in fields initially colonized that year than in fields that received an accumulated benefit from the previous year's releases and were also colonized in 1955. In 1956 records were taken in 31 fields on 11 plantations on which the Cuban fly had been the most effective for the previous 2 or 3 years. In 13 fields examined for borer-killed deadhearts in the spring the average parasitization the previous fall had been 43%. The eight fields with this average or above had a borer infestation 50% less than the five fields with below-average parasitization. Examinations made at time of harvest in all fields showed an average parasitization of 46% with a borer infestation of 19%.

Parasites of four species of stalk borers and pink bollworm bred at the Moorestown, N. J., laboratory from stock obtained from India were tested against the sugarcane borer in 1954 and 1955. A total of 27,569 parasites—10,116 of *Apanteles angaleti* Meus., 11,012 of *Bracon brevicornis* Wesm., 4,756 of *Chelonus* n. sp., and 1,685 of *Chelonus heliopae* Gupta—were released on 10 plantations and in a rice field in the Houma area. Although over 1,000 borer stages were collected in the fall and winter surveys of 1954–56 to determine possible establishment in all release fields, none of the parasites were recovered.

Agathis stigmaterus (Cress.), a borer parasite introduced from Florida, was recovered in 1956 in an area where 68 adults had been released in 1944. Three unemerged cocoons were collected while searching for other parasites. This wasp has been introduced from South America in the early 1930's and is now generally established in southern Florida. It appears to be of some economic value in borer control in that State.

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Tests of Insecticides and Application Methods against the Carrot Rust Fly in the Peat-Muck Soil of the Holland Marsh, Ontario, 1949–1954[1]

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ABSTRACT

Results of three-fifths of the tests conducted for the control of the carrot rust fly, *Psila rosae* (F.), were discarded as unreliable because of low or irregular infestations in the check plots. Control of the first generation attacking early carrots was obtained with foliage sprays of chlordane, aldrin, dieldrin, or lindane applied when the adults appeared in the field and 10 days later. The foliage was burned and the yields were lower when the plants were sprayed with a weedkiller and an insecticide combined. A preplanting soil treatment of aldrin or chlordane as a dust, granulated dust, or emulsifiable concentrate at 6 pounds of toxicant per acre gave excellent control of the second generation on late carrots; dusts of heptachlor, parathion, and dieldrin also gave excellent protection. A preplanting soil application of any one of the above insecticides to early carrots in May satisfactorily protected these carrots in the autumn. Of the several insecticides tested as foliage dusts and sprays and as seed and furrow treatments against the second generation, only parathion as a foliage spray or as a seed treatment was effective; however, this insecticide is not recommended in Ontario because of its high mammalian toxicity.

The carrot rust fly, *Psila rosae* (F.), a pest of carrots, parsnips, and other umbelliferous crops, was first recorded in Ontario in 1885. It has been reported in all the Canadian provinces except Alberta, Saskatchewan, and Manitoba. The first damage to carrots in the Holland Marsh area in Ontario, 40 miles north of Toronto, was reported in 1936, nine years after the area was drained and put into vegetable production. The Marsh is one of Canada's most concentrated vegetable-growing areas; of the total of almost 7000 acres of peat-muck soil, about 3000 acres are devoted to carrots annually. The rust fly severely damaged the carrot crop in 1946, and destroyed about 150,000 bushels, at least 30% of the crop, in 1947. The Department of Entomology and Zoology of the Ontario Agricultural College, Guelph, initiated control studies in 1947. The work was continued by the Entomology Division, Ottawa, with assistance from the College, from 1948 to 1954.

This paper describes attempts to develop effective chemical control measures for this species in the peat-muck soil of the Holland Marsh. Although much information is available on control measures for the insect attacking carrots grown in inorganic soils, there are few reports of similar work done on carrots grown in soils of high organic content. Morrison & Crowell (1952) found that wettable powders of chlordane at 4 pounds of toxicant per acre and of toxaphene at 10 pounds, applied either dry or as sprays, as preplanting soil treatments controlled the insect attacking carrots in peat soils. Wright (1957) showed that preplanting soil treatments of aldrin, dieldrin, and lindane at either 2 or 4 pounds of toxicant per acre controlled both the first and second generations of the insect on carrots grown in fen soil; aldrin at 2 pounds was the least effective; seed dressings of 50% endrin at 2 to 4 ounces per pound of seed and of lindane at 1 ounce gave only partial control of the first generation and none of the second.

Two main carrot crops, an early and a late, are grown on the Marsh each year. In normal seasons two generations of the fly occur; the first generation attacks the early crop, and the second the late. Unharvested fields of early carrots may be attacked by both generations of the insect.

GENERAL METHODS.—Experiments were conducted on early carrots sown in late April or early May and harvested in July or August, on late carrots sown in June or July and harvested in October or November, and on late-harvested early carrots sown in May but not harvested until the autumn. Carrot seed, usually of the Nantes type, was sown at 3.5 pounds per acre.

All experiments were of randomized-block or latin-square design, the number of replications varying from 3 to 6. Each plot consisted of 6 rows 25 or 50 feet long and had an area of 0.01 or 0.005 acre, respectively. An untreated row was left between adjacent plots as a buffer area. Samples of 150 or more roots were taken at harvest from the center two rows of each plot and immediately washed and examined for injury. The Farm Products Grades and Sales Act of Ontario requires that carrots for Canada No. 1 Grade be "free from insect damage affecting the appearance, or edible, or shipping quality, that cannot be removed without the loss of more than 5% of the carrot." Because of this stringent regulation any carrot damaged by the maggots was considered unsalable and the criterion of insecticidal effectiveness was whether the roots were damaged by maggots. A treatment was considered satisfactory only if it reduced the number of damaged roots by at least 60%.

Irregular infestation made it impractical to evaluate the treatments in some of the experiments. Even in sheltered areas of the Marsh, where attacks were most likely to occur, the number of damaged carrots in check plots only a few feet apart varied considerably. Infestations also fluctuated greatly from year to year; from 1949 to

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Table 1.—Effectiveness of insecticides applied as foliage sprays against the carrot rust fly in early carrots, Holland Marsh, Ont. 1949–51.

Insecticide[a]	Per Cent Infested Roots at Harvest	Per Cent Control[b]
1949, applications on June 3 and 13		
15% parathion W.P.	1	99
40% chlordane W.P.	11	85
40% chlordane E.C.	18	76
25% DDT E.C.	32	57
Untreated	74	
L.S.D. at 5% level		4.0
1950, wettable powders applied on June 6 and 16		
25% aldrin	6	71
40% chlordane	8	62
Untreated	21	
L.S.D. at 5% level		1.5
1951, wettable powders applied on June 1 and 11[c]		
25% dieldrin	1	97
25% aldrin	3	91
25% lindane	3	91
15% parathion	3	91
40% chlordane	8	77
Untreated	34	

[a] Applications at 2 pounds of toxicant per acre on each date except that 25% lindane and 15% parathion were applied at 1 pound of toxicant in 1951. W.P., wettable powder; E.C., emulsifiable concentrate.
[b] By Abbott's formula.
[c] Data not available from individual replicates.

1954 the average infestation in the check plots of early carrots varied between 1 and 40%. Similar fluctuations occurred on late carrots although this crop was usually more severely attacked. As statistical analysis showed that data from most experiments with less than 20% infestation in the check plots were unreliable, results from three-fifths of all the tests are omitted.

The experiments were discontinued in 1954, after two seasons of low infestations; in 1953 and 1954 the average infestation in the check plots was 3% in early carrots and 16% in late. In the autumn of 1954 all the rust fly larvae and most of the pupae were killed when the Marsh was flooded for 2 to 3 weeks because of a hurricane; maggots were almost impossible to find in 1955 and 1956 (Backs 1957).

Materials and Application.—The insecticides tested in the various experiments and the rates and times of application are indicated in tables 1 and 2 or in the text. All were commercial products and were applied as follows: (1) soil treatments, (2) seed or furrow treatments, (3) foliage sprays, and (4) foliage dusts.

(1) In soil treatments, sprays, dusts, or granulated formulations were applied to the soil surface before planting and disked in to a depth of 3 to 4 inches.

(2) Seed treatments were made in 1950 and 1951 by thoroughly mixing dry seed with wettable powders. In 1952, half of the seed was treated in this way but the other half was first dipped in methyl cellulose solution, which served as a sticker and caused the insecticide to form an even coating around each seed. The seed was planted on the day of treatment. In 1953 and 1954, granulated insecticides were run into the furrow at planting.

(3, 4) Foliage sprays were applied with a hand-operated compressed-air sprayer at 100 gallons of spray per acre; the dusts, with a "pump-gun" duster at various rates. Applications were made to the foliage, top of the root, and a 2- to 3-inch strip of soil on each side of the row. The first application was made when the adults first appeared in the field, on early carrots usually during the first week in June and on late carrots during the first 3 weeks of August. Since the flies were present on early carrots for about 3½ weeks and on late carrots for 4½ weeks, two applications on early carrots and three on late carrots were made at 10-day intervals. Young plants were sprayed once in early June with Agricultural Weedkiller No. 1,[5] containing one of several insecticides, in an attempt to kill the weeds and the rust fly in one operation; the weedkiller was applied at 50 gallons per acre when the carrots were in the 2- to 4-leaf stage.

Early Carrots.—Of the chemicals tested as foliage sprays, all except DDT emulsifiable concentrate gave satisfactory control of moderate or severe infestations (table 1).

In the insecticide-weedkiller experiment in 1951, the only year in which more than 20% of the untreated carrots were damaged, emulsifiable concentrates of 25% parathion, 20% aldrin, and 40% chlordane and wettable powders of 60% dieldrin and 99.5% lindane were applied at 2 pounds of toxicant in the weedkiller on June 4; adults of the rust fly were plentiful on the carrot foliage at that time and the plants were in the 2- to 4-leaf stage. All the chemicals tested except chlordane gave at least 75% control of the severe (82%) infestation. However, foliage was burned in all the treated plots although not in plots sprayed with weedkiller only. Most of the injured plants eventually recovered but gave lower yields.

Data from all experiments on soil, seed, and foliage dust treatments for early carrots were discarded because of low maggot infestations.

Late Carrots.—*Soil treatments.*—Application of insecticides to the soil before planting was the most effective control method for late carrots. Table 2 presents data showing that although satisfactory control of a severe infestation was not obtained in 1951 with wettable powders of the insecticides, the dusts tested gave excellent protection against a much lighter infestation in 1952. Granulated aldrin, heptachlor, and chlordane were very effective against a light infestation in 1953, as were emulsifiable concentrates of aldrin and chlordane in 1954.

Seed and furrow treatments.—Of the several insecticides tested, only parathion as a seed treatment gave satisfactory control in late carrots. Heavy maggot damage occurred in the untreated plots from 1950 to 1952, but the damage was relatively light in 1953. In 1950, a seed treatment on May 24 of 25% parathion wettable powder at 0.25 pound of toxicant per acre gave 72% control of a heavy (62%) infestation; parathion was significantly better than 25% lindane wettable powder at the same rate which gave only 53% control. Calomel at 3 pounds per acre was ineffective. When applied on June 19, 1951, at a slightly heavier rate than in 1950, 15% parathion wettable powder gave very poor control (24%) of an 85% infestation; wettable powders of 25% lindane, aldrin, and dieldrin at 0.5 pound of toxicant per acre, and of 40%

[5] Mineral oil (100%), Shell Oil Co. of Canada, Toronto, Canada.

chlordane at 0.75 pound, were also ineffective. Calomel at 5.5 pounds and 50% DDT wettable powder at 1 pound of toxicant per acre gave no control. In 1952, wettable powders of 25% lindane and dieldrin at 0.17 pound of toxicant per acre, of 40% chlordane at 0.2 pound, and of 20% aldrin at 0.1 pound, mixed on May 24 with either dry seed or with seed dipped in methyl cellulose solution, each gave very poor control of a severe (88%) infestation. In 1953, a furrow application on June 24 of 5% granulated chlordane at 2.5 pounds of toxicant per acre gave 62% control of a light (21%) infestation; 5% granulated aldrin and 2.5% granulated heptachlor at the same rate were both ineffective.

Foliage sprays.—Parathion was the only insecticide of the several tested that gave satisfactory control in late carrots. In 1949, three applications of 25% parathion wettable powder at either 1 or 2 pounds of toxicant per acre on August 4, 12, and 22 almost completely controlled a light (28%) infestation; but wettable powders of 25% BHC and 40% chlordane at 1 pound of toxicant per application gave only 29% and 25% control, respectively. In 1951, emulsifiable concentrates of 15% dieldrin, 40% chlordane, and 20% aldrin at 2 pounds of toxicant per acre, and of 20% lindane at 1 pound, applied on August 10, 20, and 30, gave negligible control of an 86% infestation. Similarly, wettable powders of 25% dieldrin, 50% chlordane, 20% aldrin, 25% heptachlor, and 25% Dilan applied on August 14, 24, and September 3 at 2 pounds of toxicant per acre were each ineffective against a 95% maggot infestation. Emulsifiable concentrates of 25% heptachlor, 20% aldrin, 75% chlordane, and 50% malathion, at 2 pounds of toxicant per acre on August 5, 16, and 26, were unsatisfactory against a 36% infestation in 1954.

Foliage dusts.—No insecticide tested in any of these experiments gave satisfactory control of very severe infestations. In 1950, 2.5% dieldrin and 2.5% aldrin applied on August 15, 25, and September 8 at 2½ pounds of toxicant per acre per application gave 58% and 40% control, respectively, of a 99% infestation; 5% chlordane and 5% DDT at 5 pounds of toxicant per acre, and 3% methoxychlor at 3 pounds, applied on the same dates, each gave negligible control. Dusts of 2.5% dieldrin or aldrin at 1.25 pounds of toxicant per acre, and of 5% chlordane at 2½ pounds, applied on August 22 and September 1 and 11, were ineffective against an 89% infestation in 1951. Dusts of 1% parathion at 0.5 pound of toxicant per acre, of 2.5% dieldrin, aldrin, or heptachlor at 1¼ pounds, of 5% chlordane at 2½ pounds, and of 0.6% lindane at ⅓ pound, applied on August 13 and 23 and September 2, each gave very poor control of a 99% infestation in 1952. In 1953, 5% aldrin at 2 pounds of toxicant per acre on August 4, 13, and 24 gave 51% control of a 61% infestation; 2.5% heptachlor at 2 pounds gave 46% control; 5% chlordane and 4% malathion at 2 pounds gave 31% and 17% control, respectively; 1% lindane at 2/3 pound was ineffective.

LATE-HARVESTED EARLY CARROTS.—*Soil treatments.*—The preplanting application of insecticides to the soil for carrots sown in May was the only method tested that satisfactorily controlled the second generation of the insect attacking these carrots in the autumn. In 1952, dusts of 1% lindane applied at 2 pounds of toxicant per acre on May 12, and of 5% heptachlor, 1% parathion, 10% chlordane, 2.5% aldrin, and 2.5% dieldrin applied at 6 pounds on the same date, each gave at least 90% control of a 50% infestation that developed by the end of September, the attack by the first generation having been light. In 1953, granulated formulations of 5% chlordane, 5% aldrin, and 2.5% heptachlor applied at 6 pounds of toxicant per acre on May 10 each gave at least 70% control of a severe infestation in October; 4% malathion dust at 6 pounds was ineffective.

Table 2.—Effectiveness of insecticides applied as preplanting soil treatments against the carrot rust fly in late carrots, Holland Marsh, Ont. 1951–54.

INSECTICIDE[a]	PER CENT INFESTED ROOTS AT HARVEST	PER CENT CONTROL[b]
1951, wettable powders applied on June 8[c]		
25% dieldrin	36	54
25% heptachlor	41	48
25% aldrin	43	45
15% parathion	49	38
25% lindane	50	37
40% chlordane	56	29
Untreated	79	
1952, dusts applied on May 24		
1% lindane	2	96
10% chlordane	3	94
1% parathion	3	94
2.5% aldrin	3	94
5% heptachlor	4	92
25% dieldrin	4	92
Untreated	48	
L.S.D. at 5% level		5.9
1953, dusts applied on June 26		
5% granulated aldrin	1	97
2.5% granulated heptachlor	1	97
5% granulated chlordane	2	93
4% malathion	28	3
1% lindane	28	3
Untreated	29	
L.S.D. at 5% level		8.6
1954, emulsifiable concentrates applied on June 24		
20% aldrin	0	100
75% chlordane	0	100
Untreated	31	

[a] Applications at 6 pounds of toxicant per acre except that in 1951 parathion was applied at 3 pounds and lindane at 2 pounds, and lindane at 2 pounds in 1952 and at 0.6 pounds in 1953.
[b] By Abbott's formula.
[c] Data not available from individual replicates.

Seed and furrow treatments.—In 1952, wettable powders of 20% aldrin, 25% dieldrin, 25% lindane, and 40% chlordane thoroughly mixed with dry carrot seed at 4 grams of insecticide per ounce of seed on May 14 each gave very poor control of a severe infestation in late September. Similarly, 4% malathion dust, and granulated formulations of 5% chlordane, 5% aldrin, and 2.5% heptachlor, applied in the furrow at 2½ pounds of toxicant per acre on May 10, each gave very poor control of a 50% infestation in October.

Foliage sprays.—In 1954, emulsifiable concentrates of 50% malathion, 75% chlordane, 20% aldrin, and 20% heptachlor applied at 3 pounds of toxicant per acre on June 8 and 18 gave no control of a heavy second-generation attack in September.

Foliage dusts.—In 1954, 5% aldrin, 5% chlordane,

2.5% heptachlor, and 4% malathion applied at 3 pounds of toxicant per acre on June 5 and 15 each failed to control a 30% infestation in September.

DISCUSSION.—Damage by maggots of the carrot rust fly varied considerably from year to year and the variation within experiments seriously reduced the value of the chemical control studies. The probable reasons for such variations in population are discussed by Baker *et al.* (1942), Petherbridge *et al.* (1942), and Wright & Ashby (1946a, 1946b). Because of the variation it is uncertain whether the cost of applying insecticides annually in the study area is justified by increased yields of marketable carrots, especially of early carrots which are often only lightly attacked. In many areas, cultural control measures such as delayed planting, crop rotation, planting in windswept areas, and destruction of infested roots control the first generation adequately (Whitcomb 1938, Wright & Ashby 1946a, Salkeld 1955). The studies conducted on the Holland Marsh show that the first generation of the insect can be controlled by spraying the carrot foliage and the soil around the roots with any one of several insecticides; aldrin and chlordane are especially effective. Although no reliable data were obtained on the effectiveness of soil treatments for early carrots, aldrin, chlordane, or heptachlor applied in this way would probably be effective, for each gave excellent control of the insect attacking late carrots (table 2).

Of the several insecticides tested as foliage dusts and sprays and as seed and furrow treatments, only parathion applied as a foliage spray or as a seed treatment gave satisfactory control in late carrots. This insecticide is not recommended for rust fly control in Ontario because of the hazard to the operator. The other foliage sprays and dusts failed to control the insect in August perhaps because the dense carrot foliage provides excellent protection for the fly and thorough coverage of the foliage and the soil around the roots is difficult.

Incorporating insecticides of high residual toxicity into the soil to kill the maggots as they move to the roots makes it unnecessary to apply control measures against the adults. At the Holland Marsh this control method was the most effective one tested. Also, preplanting soil applications of aldrin, chlordane, or heptachlor on early carrots in May controlled maggots attacking these carrots in the autumn. Other workers have found that soil applications of aldrin retain their toxicity to wireworms, white grubs, and some other soil insects for two years or longer (Teidjens 1952).

About 6 pounds of toxicant per acre was required to give satisfactory control of the second generation of the rust fly in the peat-muck soil of the Marsh when the insecticides were applied as soil treatments; this rate is considerably higher than that required to give the same degree of control in inorganic soils (Wright 1951, 1955, Morrison & Crowell 1952, Tiedjens 1952). The effect of incorporating this amount of insecticide into the soil on other soil arthropods and on micro-organisms was not investigated in the present experiments. No phytotoxic effects were evident from the soil treatments. Several taste tests were conducted on carrots from the experimental plots but the results were inconclusive. However, Wright (1957) found that the flavor of carrots grown on soil of high organic content was less likely to be adversely affected by insecticides than that of carrots grown on soil of low organic content.

These studies prompted the following recommendations for control of the carrot rust fly in Ontario (Ontario Department of Agriculture 1957), and in Eastern Canada (Salkeld 1955):—*Early carrots*: (1) A preplanting soil treatment of granulated chlordane or aldrin, or of heptachlor dust, at 4 pounds of toxicant per acre worked into the top 3 to 4 inches of soil: or (2) Foliage dusts or wettable powder sprays of chlordane, 2 pounds of toxicant per acre applied when the flies appear in the field and 10 days later. *Late carrots*: A preplanting soil treatment of granulated chlordane or aldrin, or of heptachlor dust, at 6 pounds of toxicant per acre worked into the top 3 to 4 inches of soil; or a wettable powder or an emulsifiable concentrate spray of any one of these insecticides at 6 pounds of toxicant.

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The Relation Between Ionization and Toxicity to Insects for Some Neuropharmacological Compounds[1]

R. D. O'Brien and R. W. Fisher[2]

Abstract

In order to study the role of ionization in the toxicity of neural poisons to insects, the authors examine the toxicity of 35 neuropharmacological agents to five insect species and the mouse. On the hypothesis that only the unionized fraction is effective in the insect, but all is effective in the mouse, toxicity ratios, mouse:insect, are calculated and compared with the observed ratios. The results confirm the hypothesis for nitrogenous anticholinesterases, and show clearly that convulsants are intrinsically ineffective against insects. With other agents, the factors are more complex.

One of the outstanding anomalies of insect nerve physiology is that, although acetyl choline is suggested to have a vital role in nerve conduction, the compound has no effect when injected into insects or applied to insect nerve preparations. Hopf (1952) showed that not only acetyl choline, but such compounds as *d*-tubocurarine, adrenaline and prostigmine were non-toxic to the locust, although very toxic to mammals. These findings suggested to Hopf that the acetyl choline system found in mammals is absent in insects, and thus "that it is doubtful whether insecticides do act as cholinesterase inhibitors."

Several recent studies have suggested that the insect nerve is relatively impermeable to ionized compounds. Hoyle (1953) demonstrated a barrier to potassium ions in the locust nerve. Colhoun (1956) showed that acetyl choline is present in excited cockroach ganglia but does not escape into a perfusate unless the ganglia are mutilated. Twarog & Roeder (1956) showed that acetyl choline effectively blocks a preparation of the last abdominal ganglion of the cockroach only if the ganglion is 'desheathed.' O'Brien (1957a) showed that although unionized esters penetrate to their esterases in a relatively undamaged whole cockroach preparation, ionized esters do not. All these observations suggested that the non-toxicity of some of the compounds that Hopf injected was due to their ionized state.

Table 1 contains the data of Hopf, and some calculations based on the following hypotheses: (a) that the insect nerve is impermeable to ionized compounds, so that only the unionized fraction of a poison is effective; (b) that the fundamental mode of action of the compounds is the same in insects and mammals; (c) that in the mammal there is no such barrier to ionized compounds. (Lewis (1954) concluded from a study of 24 sympathomimetic amines that "ionization is not important in accounting for the differences in activity" in mammals.) In table 1, the intraperitoneal toxicity to the mouse is used as an index of the mammalian toxicity. Assuming that the locust haemolymph pH was 7.0, the pK of each compound has been used to calculate the fraction unionized in the insect. For example, if a compound has a pK of 8.0, the fraction non-ionized at pH 7.0 is 0.09. Thus for each unit of poison effective in the mouse, only 0.09 unit is effective in the locust and the toxicity ratio predicted is $1/0.09 = 11$.

The agreement of prediction with observation in table 1 is good, but the data are limited, in part because of the low upper limit of toxicity attempted by Hopf.

If hypothesis (a) were valid for all insects, one would predict that all strong bases or ionized compounds would be non-insecticidal. This is not the case, as the compounds isosystox methosulfate (Fukuto *et al.* 1955), and *O,O*-diethyl *S*-β-diethylaminoethyl phosphorothiolate (Ghosh & Newman 1955) are toxic to some insects. Both show selective toxicity between different insect species. It was possible that the hypothetical ion-impermeable barrier was absent or deficient in the susceptible species, as has been proposed to account for the selective toxicity of schradan (O'Brien & Spencer 1957).

In order to test more fully the hypothesis of an ion-impermeable barrier in some insects, and to examine the possibility of variations in the barrier between Orders, several insects were examined for susceptibility to compounds of varying pK. A convenient group was that of neuropharmacological compounds. These are nearly all nitrogenous bases, and include such variously acting compounds as: ganglionic blocking agents, parasympathomimetics, anticholinesterases, convulsants and so on. The results were used to calculate the ratio of the toxicities, mouse:insect, and the ratios compared with those expected if the three hypotheses above were correct.

Cases are likely to occur in which the species tested differ in their capacities for excretion, degradation, activation and so on, of the compounds tested. Anomalous ratios will then be obtained. This work may give some indication of the extent of such differences, and hence of the validity of the present approach.

Methods.—The insects used were: house fly (*Musca domestica* L.), American cockroach (*Periplaneta americana* (L.)), large milkweed bug (*Oncopeltus fasciatus* (Dall.)), squash bug (*Anasa tristis* (DeG.)), pea aphid (*Macrosiphum pisi* (Harris) females). The mice were SWR females, 5 to 10 weeks, from Jackson Memorial Laboratories, Maine.

Injections were made intraperitoneally in the mouse; abdominally (laterally) in the cockroach and squash bug; intramuscularly into the thoracic muscle of the house fly; and into the junction of the thorax and abdomen of the large milkweed bug. Saline was used as a solvent where possible, aqueous ethanol or propylene glycol where necessary; appropriate control injections were made for all solvents, and Abbott's corrections applied (Abbott 1925). In the case of the pea aphids, for which

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Table 1.—The influence of ionization upon toxicity to the locust.[a]

Compound	Fraction Unionized at pH 7 = F	Insect LD_{50} I	Mammal LD_{50} M	Toxicity Ratio Found $\frac{I}{M}$	Toxicity Ratio Predicted $\frac{I}{F}$
Acetyl choline	$<10^{-5}$	> 960	150	> 6.4	$>10^5$
Carbaminoyl choline	$<10^{-5}$	> 160	2	> 80	$>10^5$
Acetyl β. methyl choline	$<10^{-5}$	> 160	50	> 3.2	$>10^5$
Choline	$<10^{-5}$	>1200	31	> 39	$>10^5$
Prostigmine	$<10^{-5}$	>1200	0.4	>3000	$>10^5$
d. tubocurarine	$<10^{-5}$	> 800	0.5	>1600	$>10^5$
Adrenaline	0.0016	> 800	4	> 200	630
Atropine	0.0050	> 800	250	> 3.2	200
Eserine	0.091	23	0.8	29	11
TEPP	0	1.25	0.9	1.3	1
Parathion	0	2	3	0.7	1

[a] Insect toxicity data from Hopf (1952). $LD_{10,s}$ in γ/gm.

injection was impracticable, immersion was tried, but gave a high mortality in controls. Eventually topical applications in 0.33 μl. of 25% ethanol in acetone was found to give fast evaporation and low control mortality. Only 24-hour observations could be made. Abbott's correction was applied.

The sources and forms of the compounds were as follows:

Sterling-Winthrop: Kephrine HCl (methaminoacetocatechol HCl). Cobefrin HCl (racemic 3,4-dihydroxyphenyl propanolamine HCl). Neo-synephrine HCl [l-1-(*m*-hydroxyphenyl)-2-methylaminoethanol]. Phenocaine.

Poulenc: Diparcol (*N*-diethylaminoethanol phenothiazine). Flaxedil (tri-[diethylaminoethoxy benzene]triethiodide). Homatropine methyl bromide.

Squibb: Tolserol (3-*o*-toloxyl-1,2-propanediol).

Eli Lilly: Piperocaine HCl.

Ciba: Privine HCl(α-napthyl 2-methylimidazoline).

Nutritional Biochemicals: Hordenine sulfate. Acetyl-β-methyl choline chloride. Pilocarpine nitrate. Atropine alkaloid.

Bios: Hexamethonium bromide. Decamethonium bromide. Eserine sulfate. Tetraethylammonium iodide.

Delta: Strychnine nitrate. Lobeline sulfate. Procaine HCl. Picrotoxin.

Hoffman LaRoche: Nu 683 [dimethyl carbamate of (2-hydroxy-5-phenyl benzene) trimethylammonium bromide]. Nu 1250 [*N*-*p*-chlorophenyl *N*-methyl carbamate of *m*-hydroxyphenyl trimethylammonium bromide]. Ro 3-0412 (dimethyl *m*-trimethylammoniumphenyl phosphate). Ro 3-0340 (diethyl *m*-trimethylammoniumphenyl phosphate).

Fisher: Nicotine alkaloid. Guanidine HCl. *L*-adrenaline.

Merck: Codeine phosphate.

Matheson, Coleman & Bell: Tyramine HCl.

General Biochemicals: noradrenaline.

Dr. T. R. Fukuto: Isosystox methosulfate (*O,O*-diethyl *S*-2-ethylmercaptoethyl phosphorothiolate methosulfate).

We are grateful to the following for gifts of the compounds indicated above. Dr. T. R. Fukuto; Hoffman LaRoche; Ciba; Eli Lilly; Squibb; Poulenc; and Sterling-Winthrop.

The pKa of each compound was found by titration of the salt form with alkali, using a glass electrode. It represents the pH at which the buffering effect of the salt-base pair is maximal, and is not corrected for activity coefficients. It was estimated graphically and should be considered only as good approximations of the actual thermodynamic constant.

RESULTS.—*Preliminary considerations.*—Some important factors must be considered before evaluating the results.

(1) The mammal selected for study will affect the observed results. The mouse was selected arbitrarily.

(2) The route of mammalian injection is most important. Perhaps the intravenous route is that most homologous to the insect intra-abdominal route. But the intravenous route exposes the heart very rapidly to high concentrations of compound, and one is likely to get acute effects unrelated to those obtained when equilibrium distribution is attained. The intraperitoneal route seemed reasonably comparable and has therefore been used.

(3) The time of observation of mortality was particularly critical in the insect. The LD_{50} at 48 hours was almost always less than that at 24 hours. The criterion of death was that there be no movement or response to stimuli: at 24 hours, there were often many insects with very faint occasional tremors in, say, one leg. These never recovered. At 48 hours one had a clearer distinction between live and dead insects, since there were fewer in this near-death state. For this reason the 48 hour LD_{50} was used in the calculations. But as the 24-hour value is commoner in the literature, we have included it also for the sake of comparing our data with that of other workers. The use of the 48-hour value will often give toxicity ratios, mammals:insects, several times lower than would the 24-hour value.

In the case of the mouse, the results were little affected when observation times were greater than 3 hours. In practice, the 24-hour observation was used. In no case did animals succumb later if they had survived for 24 hours.

(4) Often the predicted ratios were considerably higher than the observed. This was inevitable in the cases of the high toxicity ratio where the toxicity to the mouse (nearly always the more susceptible species) was low. Thus if the predicted ratio is 200, and the toxicity to the mouse is 100 γ/gm. the predicted insect toxicity is $200 \times 100 = 20{,}000$ γ/gm. In fact, almost any compound (especially a salt) injected at 5,000 or 10,000 γ/gm. will be

toxic; non-specific side effects become significant at such levels. Thus there is an upper limit on the possible toxicity, and the observed ratio will fall below the predicted. For instance, in the quaternary compounds, the predicted ratio is 10^5; but with low mouse toxicity, ratios greater than 50 or 100 cannot be expected in such cases. Since we were not interested in non-specific effects, injections were seldom made at levels above 2,000 γ/gm. In many cases solubility limitations did not permit amounts as great as this to be injected.

(5) Because the pea aphids were treated topically rather than by injection, an additional possible barrier, the integument, stands between the compounds and their site of action. Alternatively, certain susceptible sites might be more available to compounds applied topically, as suggested by Stegwee (1951). Due caution must be used, therefore, in evaluating the aphid data.

House fly and cockroach (Table 2).—Local anaesthetics: the results do not oppose the hypotheses that (a) the insect nerve is ion-impermeable, (b) the mode of action is the same in mammals and insects, (c) the mammalian system is ion-permeable. The low toxicity to the mouse precludes a more useful statement.

Ganglionic blocking agents: the results for nicotine, labeline and hexamethonium are in accordance with the hypotheses, those for hordenine and tetraethylammonium are not.

Neuro muscular inhibitors: the results are acceptable if

Table 2.—Toxicity of neurotoxic compounds to the mouse, house fly and cockroach.

				House Fly			Cockroach		
Compound	pKa	Predicted Toxicity Ratio[a]	Mouse LD_{50}	LD_{50} 24-hr.	LD_{50} 48-hr.	Observed Toxicity Ratio[c]	LD_{50} 24-hr.	LD_{50} 48-hr.	Observed Toxicity Ratio[c]
Local anaesthetics									
Procaine	9.1	127	200[b]	>2500	>2500	> 13	>2000	>2000	> 10
Phenocaine	7.6	5	—	740	740	—	>1000	870	—
Piperocaine	8.6	41	120[b]	>2000	>2000	> 17	>1500	>1500	> 14
Ganglionic blocking agents									
Nicotine	8.1	14	20	830	500	25	800	560	28
Lobeline	8.0	11	40	1700	1500	37	>2000	>2000	> 50
Hordenine	9.8	625	*ca.* 1000	>2500	>2500	> 2.5	1600	500	0.5
Tetraethyl ammonium	Q[d]	> 10^5	65	2800	2500	38	2300	800	12
Hexamethonium	Q	> 10^5	60	>2500	—	[> 42]	>2000	>2000	> 33
Neuromuscular inhibitors									
Decamethonium	Q	> 10^5	3	2000	930	310	>1000	>1000	> 330
Flaxedil	Q	> 10^5	9	>2000	1500	167	>1000	>1000	> 110
Tolserol	NB[e]	1	600	1500	1500	2.5	>1000	>1000	> 1.7
Parasympatholytics									
Atropine	9.3	200	250	1500	200	0.8	>1000	>1000	> 4
Homatropine	Q	> 10^5	60	>2500	>2500	> 42	>2000	>2000	> 33
Parasympathomimetics									
Acetyl choline	Q	> 10^5	150	>2000	>2000	> 13	>2000	>2000	> 13
Acetyl β-methyl choline	Q	> 10^5	50	>2500	>2500	> 50	>2000	>2000	> 40
Pilocarpine	7.2	2.6	100	1700	400	4	750	900	9
Guanidine	11.6	> 10^4	175[b]	2000	1300	7.4	—	—	—
Carbaminoyl choline	Q	> 10^5	2	190	130	65	790	590	295
Narcotic									
Codeine	8	11	*ca.* 100	>2500	>2500	> 23	>2500	>2500	> 25
Sympathomimetics									
Adrenaline	9.8	625	4	—	—	—	2000	800	200
Noradrenaline	9.1	112	50	>2500	>2500	> 50	2000	2000	40
Neosynephrine	9.7	500	>100	>2500	>2500	?	>2000	>2000	?
Cobefrine	9.3	200	—	>2500	>2500	—	>2000	>2000	—
Kephrine	9.6	400	>100	>2500	>2500	?	>1000	>1000	?
Privine	10.5	3200	50[b]	>1000	>1000	> 20	—	—	—
Tyramine	10.4	2500	>100	—	—	—	>2000	1100	?
Anticholinesterases									
Nu 1250	Q	> 10^5	0.3	590	500	1700	890	500	1700
Nu 683	Q	> 10^5	1.0	560	560	560	790	630	630
Ro 30412	Q	> 10^5	0.4	107	107	270	>1000	630	1570
Ro 30340	Q	> 10^5	8	—	—	—	>1000	>1000	> 125
Isosystox methosulfate	Q	> 10^5	3	100	15	5	300	300	100
Eserine	8.0	11	0.8	200	93	116	> 250	80	100
Convulsants									
Strychnine	7	2	1.4[b]	>1000	>1000	> 710	>1000	>1000	> 710
Picrotoxin	NB	1	4.5	1300	710	> 160	> 500	> 500	> 110
Anticonvulsant									
Diparcol	7.1	2.3	250	630	630	2.5	100	660	2.6

[a] Ratio = 1/(fraction non-ionized at pH 7).
[b] Rat.
[c] Ratio = (48 hr. insect LD_{50})/(mouse LD_{50}).
[d] Quaternary.
[e] Non-basic.

Table 3.—Toxicity of neurotoxic compounds to the milkweed bug, squash bug and pea aphid.

Compound	Predicted Toxicity Ratio	Milkweed Bug			Squash Bug			Pea Aphid	
		LD_{50} 24-hr.	LD_{50} 48-hr.	Observed Toxicity Ratio[a]	LD_{50} 24-hr.	LD_{50} 48-hr.	Observed Toxicity Ratio[a]	LD_{50} 24-hr.	Observed Toxicity Ratio[b]
Procaine	127	2000	1600	8	>2000	>2000	> 10	2131	11
Phenocaine	5	800	600	—	—	—	—	—	—
Piperocaine	41	>2500	2500	21	>2500	>2500	> 21	742	6.2
Nicotine	14	1200	145	7.3	430	500	25	—	—
Lobeline	11	1500	1100	28	—	400	10	107	2.7
Hordenine	625	>2500	>2500	> 2.5	2000	2000	2	—	—
Tetraethyl ammonium	> 10^5	>2500	>2500	> 38	3000	3000	46	2440	38
Hexamethonium	> 10^5	—	—	—	>2000	>2000	> 33	7410	123
Decamethonium	> 10^5	>1000	125	42	>1000	>1000	>330	—	—
Flaxedil	> 10^5	>2500	>2500	> 280	>2000	2000	220	2140	238
Tolserol	1	360	63	0.1	>1000	800	1.3	3300	55
Atropine	200	—	—	—	> 500	950	3.8	1650	6.6
Homatropine	> 10^5	>2500	>2500	> 42	>2000	>2000	> 33	2350	39
Acetyl choline	> 10^5	>2000	>2000	> 13	>2000	>2000	> 13	710	4.7
Acetyl β-methyl choline	> 10^5	>2500	>2500	> 50	>2500	2500	50	—	—
Pilocarpine	2.6	25	10	0.10	40	7	0.07	4120	41
Guanidine	> 10^4	>2000	>2000	> 11	>2000	>2000	> 11	—	—
Carbaminoyl choline	> 10^5	—	—	—	800	650	325	—	—
Codeine	11	>2500	>2500	> 25	—	—	—	—	—
Adrenaline	625	>2500	2000	500	—	—	—	—	—
Noradrenaline	112	>2500	>2500	> 50	>2500	>2500	> 50	330	6.6
Neosynephrine	500	>2500	>2500	?	>2000	>2000	?	—	—
Cobefrine	200	>2500	2000	—	—	—	—	[c]	—
Kephrine	400	>2500	>2500	?	>5000	>5000	?	[c]	—
Privine	3200	500	150	3	c.1000	450	9	—	—
Tyramine	2500	—	—	—	>2500	>2500	?	—	—
Nu 1250	> 10^5	> 250	200	670	300	220	730	924	3100
Nu 683	> 10^5	>1000	200	200	> 500	400	400	742	742
Ro 30412	> 10^5	> 500	> 500	>2000	350	150	370	—	—
Ro 30340	> 10^5	800	660	83	600	170	21	—	—
Isosystox methosulfate	> 10^5	> 500	100	33	43	35	12	41	14
Eserine	11	400	63	79	8	3	3.7	3.6	4
Strychnine	2	>1000	>1000	> 710	> 500	> 500	>360	[c]	—
Picrotoxin	1	1000	400	89	>1000	>1000	>220	1980	440
Diparcol	2.3	1600	1200	4.8	>1000	>1000	> 4	1150	4.6

[a] Ratio = (48-hr. insect LD_{50})/(mouse LD_{50}).
[b] Ratio = (24-hr. insect LD_{50})/(mouse LD_{50}).
[c] Insoluble in the solvent mixture employed.

one allows the validity of consideration (4) above in the cases of decamethonium and Flaxedil with the house fly.

Parasympathominetics: the results for pilocarpine are very encouraging; the others are acceptable if consideration (4) above is applicable to guanidine and (in the case of the house fly) to carbaminoyl choline.

Narcotic: results inconclusive.

Sympathomimetics: results are either inconclusive, due to the low mammalian toxicity, or in reasonable agreement with the hypotheses.

Anticholinesterases: the results for the Roche and Nutley quaternaries give good support for the hypotheses. The results for isosytox methosulfate (fully ionized, but at the sulphur rather than the nitrogen), is radically out of accordance with prediction, especially for the house fly. The eserine results are rather dubious, as the observed ratio is 10 times that predicted.

Convulsants: clearly hypothesis (b)—that the mode of action is identical in insects and mammals—is completely incorrect.

Anticonvulsant: there is extraordinarily good agreement of prediction with observation.

Large milkweed bug, squash bug and pea aphid (Table 3).—Table 4 shows an analysis of the results, along with those for the house fly and cockroach, for comparison. There is perhaps rather less uniformity in the response of the large milkweed bug and squash bug to a given class of compounds, which in itself suggests that the mode of action is not as in the mammal. In the pea aphid, agreement of prediction and observation is extremely poor;

except for the cases of Tolserol, pilocarpine and picrotoxin, this poor agreement is caused by the unexpectedly high toxicity of the compounds. This offers some support for the idea that the nervous system of the pea aphid is not so well protected as that of the other insects studied.

DISCUSSION.—No attempt has been made in this study to duplicate the very large number of observations in the literature on the toxicity to insects and mammals of organophosphorus insecticides. Since the vast majority of these are unionizable, a toxicity ratio, mammals:insects, of 1 would be predicted, if all three hypotheses (a, b, c, above) were correct. Variability arises in some cases because of marked species variation in the capacity to activate or deactivate the organophosphates (e.g. malathion, March *et al.*, 1956, O'Brien 1957b) or of species variation with respect to penetrability of the nerve cord (O'Brien & Spencer 1957). Table 5 shows data calculated from the observations from Krueger & Casida (1957) using only their observations upon insects identical with or closely related to species in the present study. Only in six cases out of the 45 reported in table 5 do the mammal:insect toxicity ratios exceed the value of 10. In general the unionized organophosphates are at least as toxic to insects as to mammals.

Richardson & Shepard (1930) showed that the speed of toxic action of nicotine solutions to larval *Culex pipiens* was directly related to the concentration of undissociated nicotine molecules. Craig (1933) and Craig & Richardson (1933) found a direct correlation between the dissociation constants and the toxicities to *Apis nimicis* of a series of α-substituted *N*-methylpyrrolidines. These results (which were in fact otherwise interpreted) are in agreement with the existence of an ion-impermeable barrier in these insects.

Extensive studies have been made by Turner & Saunders (1946) (spraying aphids) and Turner *et al.* (1948) (injecting large milkweed bugs) upon the relative toxicity of nicotine and nicotinium compounds, and upon the effect of quaternerization in other heterocyclic nitrogen bases. Their results show that the effect of an ionizing group depends upon the molecule into which it is introduced. In general, the transformation of a nicotine-like compound into a closely related nicotinium one (e.g. nicotine to a methyl nicotinium salt) always produced a sharp drop in toxicity. Nevertheless, certain quaternary heterocyclic compounds were even more toxic than nicotine (e.g. 2-methyl*iso*-quinolinium iodide), and "quaternary compounds of the coal tar bases and their derivatives were relatively much more toxic than the parent compounds," the parent compounds being pyridine, piperidine, quinoline, etc. In the absence of other evidence, it seems quite possible that the simpler heterocyclic bases may act in ways quite unlike that of nicotine. Turner *et al.* (1948) point in their introduction to some evidence for the toxic nicotinium salts having a different mode of action from nicotine, although after examination of their large amount of data, they conclude that the case is not proven. In some of the large and complex molecules particularly one has to bear in mind the possibility of metabolism in the insect to simpler toxic molecules without the quaternary grouping.

Kolbezen *et al.* (1954) showed that in a large number of carbamate anticholinesterases, the presence of a quaternary group, whilst raising the anticholinesterase activity and mammalian toxicity, eliminated insect toxicity.

Table 4.—Agreement of predicted and observed mammal: insect toxicity ratios.[a]

	House Fly	Cockroach	Large Milkweed Bug	Squash Bug	Pea Aphid
Local anaesthetics	B	B	D, A	B	D
Ganglionic blocking agents	B	D	A, B	A, D	D, A
Neuromuscular inhibitors	B	B	D	A	D, E
Parasympatholytics	D, B	B	B	E, B	E
Parasympathomimetics	A, D	A	B, E	B, E	E
Narcotic	C	C	B	—	—
Sympathomimetics	B, C	A, C	A, B, C, E	B, C, E	E
Anticholinesterases	B, D, E	A, D	A, B, D	A, E	A, E
Convulsants	E	E	E	E	E
Anticonvulsants	A	A	A	B	A

[a] A: In good agreement with hypotheses that (a) insect nerve is ion-impermeable; (b) mode of action is identical in mammals and insects; (c) ionization not important to action in mouse (i.e. predicted and observed ratios agree).
B: Results do not oppose hypotheses (i.e. observed ratio is ">x," and x is not greater than the predicted ratio).
C: Results inconclusive (i.e. observed ratio could not be calculated).
D: Results doubtful (i.e. about 10x difference between prediction and observation).
E: Results clearly oppose hypotheses (>10x difference between prediction and observation).

Perhaps the most disturbing anomaly, upon which no light is thrown by this study, is that the following three ionizable organophosphates are toxic to some insects: *O,O*-diethyl *S*-ethyl-2-mercaptoethyl phosphorothionate methosulfate (isosystox methosulfate); *O,O*-diethyl *S*-2-diethylaminoethyl phosphorothiodate (Chipman R-6199); and the methosulfate of R-1699. However, Fukuto (1957) states that for isosystox and R-1699 "the comparative contact toxicities of the methosulfates, to various insects . . . is much lower than the parent compounds and can only be attributed to slow penetration of the positively charged ions." Fukuto then goes on to point out that in Chipman R-6199 itself, the free amine (and its hydrated form) could penetrate, although the salt form could not.

This raises the point of the validity of applying hypothesis (a) above to those compounds which, when in solution, have an ionized and a non-ionized species in equilibrium. Consider two compartments, A and B, separated by a membrane which is fully permeable to unionized solute, but impermeable to ions. If to A one adds a dissociable compound whose pK is such that at the prevailing pH it is 99% ionized, then diffusion of the compound across the membrane will be extremely slow, since the effective concentration of the compound in A (from the point of view of diffusion) is only 1% of the total added. Nevertheless diffusion will occur, and in B the same equilibrium will be set up; eventually there will be the same concentration of solute in B as in A. Let us now suppose that this model applies to the insect: that A is the body cavity, B is the nervous system. What influence would ionization have upon the effective toxicity of neurotoxic agents injected into A? Let us consider three cases: (1) If the agent were stable and had a cumulative action, one would not expect ionization to be important, since it would be the final equilibrium concentration that would decide the toxicity; (2) If the agent had a cumulative action, but was rapidly metabolized in or excreted from either A or B, then ionization, by retarding the rate of achievement of equilibrium and thus giving time for

Table 5.—Toxicity ratios, mammals:insects, of non-ionized organophosphates.[a]

	Macrosiphum pisi	*Musca domestica*	*Blattella germanica*
Dipterex	0.05	0.03	0.08
Phosdrin	1.0	1.3	8.7
Para-oxon	1.0	1.0	1.5
Diethyl 2,4,5-trichlorophenyl phosphate	0.02	0.03	0.4
Pyrazoxon	4.3	21	10
O-ethyl *O*-*p*-nitrophenyl phenylphosphate	2.0	2.5	3.3
DFP	3.6	8.6	114
O-ethyl *O,O*-bis(*p*-nitrophenyl) phosphorothioate	>6	?	?
O-2-(ethoxy)ethyl *O,O*-diethyl phosphate	.04	.06	0.09
Am. Cyanamid 12008	0.4	1.0	7.5
S-(2-oxoureidoethyl)*O,O*-diethyl phosphorodithioate	0.06	13	20
Methyl parathion	0.08	0.25	.33
Malathion	0.0008	0.03	0.12
Schradan	1.1	>110	>110
Mipafox	0.3	0.93	0.3

[a] Results calculated from data of Krueger & Casida (1957). Insects treated topically. Mammal was the white rat, injected subcutaneously.

detoxifying processes to act, would reduce the toxicity of the compound; (3) If the agent was non-cumulative in its action and was rapidly detoxified in A or B, then its effect would be determined by the concentration achieved in B soon after injection; in this case it would be true that only the unionized fraction (as calculated from the pK of the compound and the pH of A) would be effective.

In the results reported above, the effects of the poisons were always slow in appearing; as a correlate, the 48-hour LD_{50}'s were often lower than those at 24 hours. This suggests that case (3) in the preceding paragraph does not hold, unless of course the target site is promptly poisoned and it is only the effects of this poisoning that are slow in appearing.

In view of the facts that with unionized organophosphates (table 5) the toxicity ratio (mammals:insects) is in the order of 1, but that with ionized compounds (table 4) the results fall somewhere between those expected from cases (1) or (3), the most satisfactory hypothesis is that case (2) is the prevailing one in the case of the compounds and insects studied in this paper.

CONCLUSIONS.—It is unfortunate that this work has not been able to throw definite light on the possibility of there being an adrenergic system in insects. It can only be said that sympathomimetic agents are non-toxic to all insects studied, but that this could as well be caused by the ionized state of the compounds as by an intrinsic difference in their potential mode of action.

The results on neuromuscular blocking agents are more hopeful; Tolserol, presumably because of its non-ionized state, has a similar toxicity for insects and the mouse. It is therefore a suitable experimental agent for study with insects. The more familiar agent, decamethonium, is quite unsuitable.

The comparison of the effects of the compounds studied upon the five insect species do not show a clear-cut variation in sensitivity for one species, nor do the results for the squash bug, large milkweed bug and pea aphid clearly suggest that these insects have a less effective barrier to ionized compounds than the cockroach and house fly, as suggested in the introduction.

This work started with the problem of the relative importance of cholinesterase and acetyl choline (and its derivatives) in insects and mammals. As a contribution towards the problem, it can be stated that (1) The introduction of a quaternary nitrogen into an anticholinesterase compound greatly increases the mammal:insect toxicity ratio. The effect is most marked in the house fly, cockroach and pea aphid (minimum mammal:insect toxicity ratio = 270) and is in some cases less in the large milkweed bug and squash bug (minimum ratio = 21). For some reason the introduction of an ionized sulphur group has a much smaller effect (ratios range from 5 to 100 in the five insects studied). (2) In the parasympathomimetic group of compounds, all fully ionized compounds (such as acetyl choline) have very low toxicity to insects, and usually a high mammal:insect toxicity ratio. The partially ionized pilocarpine behaves in house fly, cockroach and pea aphid as if only the non-ionized fraction were effective; but in the milkweed and squash bugs the toxicity is unexpectedly high, in fact presenting the unusual case (among the compounds and insects studied in this paper) of being more toxic than to the mouse.

The above two conclusions suggest that the failure of acetyl choline and similar ionized compounds to poison insects, or (in the case of atropine) to relieve organophosphorus poisoning, is fully attributable to their ionized state, and does not invalidate the conclusions that the choline acetylase-acetyl choline-cholinesterase system is present in and vital to insects, and that interference with it can be lethal (Spencer & O'Brien 1957).

It is certain that the convulsants are almost totally ineffective against insects, and that this is not an ionization phenomenon. Their mammalian toxicity is probably due to an asphyxiation effect, and Grollman (1954) states: "In frogs, where the breathing can be dispensed with for long periods, the alternation of convulsions and periods of quiescence may continue for hours or days." Probably insects are unaffected because of the nature of their respiratory process, which involves diffusion rather than obligatory active ventilation. It is of course true that organophosphates also may kill mammals by asphyxiation, but in this case other factors are also involved even if asphyxiation is prevented (Barnes 1953).

In view of the above, it is curious that the anticonvulsant Diparcol should give such excellent agreement between prediction and observation. This served to illustrate the difficulties of classification of these neurotoxic compounds: the fact that a compound has a certain primary pharmacological action does not mean necessarily that at high doses its toxic effect may be due to some totally different effect. It is probably safe to say of Diparcol that it kills insects and mammals in the same way; and that the susceptible site is protected from the ionized form in insects. It is quite certain that the differential toxicity of neurotoxic compounds is not to be explained solely on the basis of ionization, but it is equally certain that ionization plays a most important role.

In a more general way the results indicate the difficulty of interpreting toxicological or pharmacological experiments with insects when ionizable compounds are involved; this is the more regrettable because most of the compounds affecting mammalian nervous and muscular systems are amines. The selection or synthesis of compounds which affect such systems in the mammal, yet have pK's of 8 or below, will be necessary before experi-

ments of this kind can be used to deduce information about the insect nervous system.

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Residues Resulting from Applications of Demeton to Cabbage and Celery[1]

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ABSTRACT

The residue data accumulated over a 3-year-period on cabbage treated with demeton at Sanford, Florida, at transplanting time indicate that under some conditions dosages of more than 0.5 pound active demeton per acre may result in residues greater than the established tolerance at harvest time. However, cabbage treated in this manner during the 1955–56 growing season with 0.75, 1.00 and 1.25 pounds active demeton per acre contained residues of less than 0.75 p.p.m. 66 days after the application. Celery treated in a similar manner during the 1955–56 growing season with 1.25 pounds active demeton per acre was found to be carrying residues of 0.72 p.p.m. 100 days after the application. Lower dosages produced proportionately lower residues. Demeton residues in both celery and cabbage which had been sprayed at 21-day intervals with 0.25 pounds active demeton were less than 0.75 p.p.m. 21 days after the last application with the one exception of 0.8 p.p.m. reported by the Pittsburgh Coke and Chemical Company laboratory for the 1954–55 samples of cabbage.

The use of systemic insecticides for the control of crop pests has received much attention for several years. Early experiments conducted in England began in 1947. For several years Ripper *et al.* (1950) treated many crops with bis (bis dimethylamino phosphonous) anhydride. Their work, as well as more recent experiments, has shown that systemic insecticides have distinct advantages to growers over the conventional insecticides for the control of many insects.

Using schradan and demeton, Reynolds *et al.* (1953)

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[2] The authors are indebted to Mr. Louis Steflik for assistance with the field preparation of the samples.

tested the effectiveness of these materials on cabbage against the cabbage aphid, *Brevicoryne brassicae* (L.). Seed soil drench and spray treatments with both materials gave excellent control for several weeks. Working in abandoned cabbage fields, Wene & White (1952), obtained good control of the cabbage aphid for a 2-week period with schradan applied at the rate of 0.38 pounds in 100 gallons of water. Dowdy & Sleesman (1952) found that the residual effectiveness of both schradan and demeton is dependent upon the amount of active ingredient applied to the plant. In Wisconsin, Apple & Martin (1955) applied demeton to garden peas at different stages of maturity at dosages of 1, 2, 4 and 8 ounces of actual demeton per acre. Plants within 3 weeks of harvest were protected from the pea aphid, *Macrosiphum pisi* (Harr.) by single applications of the two lower dosages. Single applications of the 4- and 8-ounce dosages to younger plants protected these younger plants for about 18 days. Harvested peas from the four dosage levels which had been canned and frozen contained residues of less than 0.3 p.p.m.

At the Central Florida Experiment Station demeton was applied to cabbage during three growing seasons, and to celery during two growing seasons. The applications were made by placing the demeton in the transplant water, and by foliage spraying. Residues resulting from these treatments are reported in this paper.

MATERIAL AND METHODS.—The demeton used for all of the treatments was formulated as an emulsifiable concentrate containing 2 pounds of active ingredient per gallon.

In the central Florida area, water at the rate of approximately 1100 gallons per acre is applied to the newly transplanted seedlings of both cabbage and celery. At the spacing usually used for cabbage, this is equivalent to about ½ pint of water per cabbage plant. The desired amount of demeton was diluted with water for the drench treatments and applied to cabbage during the 1953–54 and 1954–55 growing seasons at the rate of ½ pint of the dilute solution per plant. During the 1955–56 growing season for the drench treatments applied to both cabbage and celery, the desired amount of demeton was diluted to be applied at the rate of 1100 gallons per acre. The proportionate amount of this dilute solution was applied in a band about 5 inches wide to the entire length of the row by means of a sprinkling can after the plants had been set.

The foliage applications were made with a power sprayer equipped with a three-row boom having five hollow-cone nozzles per row. The sprayer was driven at approximately 3 miles per hour with a pump pressure of approximately 250 p.s.i. and the spray was applied at the rate of 90 gallons per acre. Single spray applications were made 2 weeks after transplanting. For treatments requiring more than one application the first application was made 2 weeks after transplanting, and subsequent applications were made at 21-day intervals during the 1954–55 season, but varied slightly from the planned 21-day interval during the 1955–56 season (see table 4). Triton B-1956 at the rate of 4 ounces per 90 gallons of spray was added to all of the foliage sprays.

The plots were 0.01 acre in size. The cabbage plants were set 12 inches apart and the celery plants were spaced 5 inches apart in the row. Rows for both the cabbage and celery were 30 inches apart. A 15-foot alleyway separated each of the four blocks. Cultural and fertilizer practices common to the area were followed. Copenhagen market cabbage and Florida pascal celery were the varieties used.

Samples for residue determinations consisted of 10 plants taken at random from the center row of each three-row plot. In conformity with the commercial practice the cabbage heads were cut with four to seven wrapper leaves and the celery was stripped of the outer petioles and topped to the standard 18-inch length in the field. In the laboratory one-quarter of each cabbage head with attached wrapper leaves was chopped into small irregular pieces. The whole celery plant was chopped into pieces about 1 inch in length. The chopped samples were placed in a mixing drum and thoroughly mixed. After mixing, 1000-gram subsamples were placed in pliofilm bags and stored at 0° F. Duplicate samples from the 1953–54 and 1954–55 experiments were shipped to the Pittsburgh Coke and Chemical Company Laboratory and to the Main Station Residue Laboratory at Gainesville for residue determinations. Residue determinations on the 1955–56 samples were made at the Main Station Laboratory only.

The Pittsburgh Coke and Chemical Company Laboratory utilized the cholinesterase inhibition method as described by Hensel *et al.* (1954) for the determination of demeton residues. This method was also followed with slight modifications at the Gainesville Laboratory for the 1953–54 and 1955–56 samples.

In general, the Gainesville Laboratory used the technique described below in sample preparation and analysis: A 150-gram segment was cut from the original 1000-gram gross sample, accurately weighed, and the remainder of the sample returned to the deep-freeze immediately. The 150-gram sample was allowed to thaw completely in a pint mason jar and placed in a Hamilton Beach blendor after 75 ml. of water had been added to aid the cutting action. After 3 minutes of blending, the macerate was squeezed through four thicknesses of cheesecloth and the filtrate saved. The 1953–54 and 1955–56 samples were squeezed by hand. In an effort to obtain uniform amounts of filtrate the 1954–55 samples were placed in a Carver Press and after thawing, were subjected to 10,000 pounds pressure. By subjecting all of the samples to the same pressure, a reproducible volume of clear filtrate was recovered. The extensive cleanup procedure of the bulky press parts, required between samples to avoid contamination, consumed so much time that the hand pressure method of separating the filtrate was again used for the 1955–56 samples. For the 1954–55 and 1955–56 samples the ratios of blood plasma, buffer, acetylcholine chloride and sample aliquot were varied slightly in an effort to get an optimum delta pH change.

RESULTS AND DISCUSSION.—Demeton was applied to cabbage at varying rates in the water used at transplanting during each of the 3 years in which this study was conducted. At the dosages used during the 1953–54 and 1955–56 growing seasons residues of demeton were well below the permissible tolerance of 0.75 p.p.m. in mature cabbage 79, 66, and 73 days after application (table 1). Furthermore, the 1953–54 data from the two laboratories agree within a narrow range. The residues reported by

Table 1.—Residues in cabbage after one application of demeton by the soil drench method.

Pounds/ Acre Applied	Dates of Application	Dates of Harvest	Days from Application to Harvest	Analyses for Residues in p. p. m. at Pittsburgh	Analyses for Residues in p. p. m. at Gainesville
		1953–54 Growing Season			
0.62	Jan. 11	April 1	79	0.2	0.28
1.25	Jan. 1	April 1	79	0.2	0.36
		1954–55 Growing Season			
0.50	Dec. 1	Feb. 9	70	0.52	1.00
1.00	Dec. 1	Feb. 9	70	2.22	5.14+
1.50	Dec. 1	Feb. 9	70	2.72	5.14+
		1955–56 Growing Season			
0.75	Nov. 21	Jan. 12	52		1.97
	Nov. 21	Jan. 19	59		1.35
	Nov. 21	Jan. 25	66		0.68
	Nov. 21	Feb. 2	73		0.37
1.00	Nov. 21	Jan. 12	52		2.17
	Nov. 21	Jan. 19	59		1.58
	Nov. 21	Jan. 25	66		0.72
	Nov. 21	Feb. 2	73		0.40
1.25	Nov. 21	Jan. 12	52		2.13
	Nov. 21	Jan. 19	59		1.37
	Nov. 21	Jan. 25	66		0.67
	Nov. 21	Feb. 2	73		0.36

Table 3.—Residues in cabbage resulting from foliage applications of demeton.

Pounds/ Acre per Application	Dates of Application	Dates of Harvest	Days from Last Application to Harvest	Residues in p. p. m. Pittsburgh	Residues in p. p. m. Gainesville
		1953–54 Growing Season			
0.50	Jan. 27	April 1	64	0.2	0.22
1.00	Jan. 27	April 1	64	0.4	0.25
		1954–55 Growing Season			
0.50	Dec. 2	Feb. 9	49	0.25	0.62
1.00	Dec. 22	Feb. 9	49	2.95	5.14+
2.72[a]	Nov. 8	Jan. 26	14	2.90	6.43+
	Nov. 12	Feb. 2	21	2.60	6.64+
	Dec. 22	Feb. 9	28	1.70	2.30
		1955–56 Growing Season			
0.25	Dec. 2	Jan. 2	0		7.81
	Dec. 22	Jan. 25	7		5.84
	Jan. 12	Feb. 2	14		1.95
		Feb. 9	21		0.62
0.50	Dec. 2	Jan. 12	0		11.92
	Dec. 22	Jan. 25	7		16.49
	Jan. 12	Feb. 2	14		3.04
		Feb. 9	21		0.75

[a] First application made in seedbed (see Table 5).

the Pittsburgh Coke and Chemical Company Laboratory were considerably lower than those reported by the Gainesville Laboratory for the three dosage levels applied during the 1954–55 growing season. Only the residue from the ½-pound (per acre) dosage as determined by the Pittsburgh Coke and Chemical Company Laboratory was below the permissible tolerance level. In the Gainesville Laboratory, determinations of the high residue levels were not made after it was found that these, indicated by a plus sign, were considerably in excess of the permissible level.

The rather wide differences between the data reported by the Pittsburgh Coke and Chemical Laboratory and the Gainesville Laboratory for the 1954–55 samples of both cabbage and celery are at least partially attributed to the following factors: (1) although every effort was made to avoid thawing of the samples in transit some thawing probably did occur in the samples shipped by air express to Pittsburgh; (2) the use of the Carver Press in the Gainesville Laboratory; (3) a new standard curve was run each day by the Gainesville Laboratory to compensate for fluctuations in plasma and enzyme activity, whereas daily standard curves were not constructed by the Pittsburgh Laboratory.

Table 2.—Residues in celery after one application of demeton by the soil drench method during the 1955–56 growing season.

Pounds/ Acre Applied	Dates of Application	Dates of First Harvest	Residues (P. P. M.) at Intervals (Days) After Application Indicated 93	100	107	114
0.75	Dec. 5	March 8	0.70	0.43	0.20	—
1.00	Dec. 5	March 8	.84	.62	.33	—
1.25	Dec. 5	March 8	.91	.72	.52	0.41

Demeton was applied to celery by the soil drench method at transplanting time during only one growing season, 1955–56. Residue data given in table 2 indicate that, at the 0.75-pound dosage, detoxification to below the 0.75 p.p.m. level took place in less than 95 days while

Table 4.—Residues in celery resulting from foliage applications of demeton.

Pounds/ Acre per Application	Dates of Application	Dates of Harvest	Days from Last Application to Harvest	Residues in in P. P. M. Pittsburgh	Residues in in P. P. M. Gainesville
		1954–55 Growing Season			
0.25	Dec. 22	Feb. 23	0	12.9	5.2+
	Jan. 12	Feb. 24	1	7.4	4.2
	Feb. 2	Feb. 26	3	4.3	2.9
	Feb. 23	March 2	7	3.5	1.2
		March 9	14	2.3	0.7
		March 16	21	0.8	0.5
		March 23	28	0.4	0.3
0.50	Dec. 22	Feb. 23	0	22.2	5.2+
	Jan. 12	Feb. 24	1	12.4	5.2+
	Feb. 2	Feb. 26	3	11.1	5.2+
	Feb. 23	March 2	7	7.8	3.1+
		March 7	14	5.9	1.8
		March 16	21	2.2	1.1
		March 23	28	1.4	0.9
		1955–56 Growing Season			
0.25	Feb. 13	March 8	0		6.01
	Jan. 6	March 15	7		1.43
	Jan. 25				
	Feb. 14	March 22	14		0.80
	March 8	March 29	21		0.35
0.50	Dec. 13	March 8	0		8.25
	Jan. 6	March 15	7		2.06
	Jan. 15				
	Feb. 14	March 22	14		1.30
	March 8	March 29	21		0.66

Table 5.—Climatological data for the months cabbage and celery were grown during three growing seasons at Sanford, Florida.

Month	1953–54			1954–55			1955–56		
	Mean Max.[a]	Mean Min.	Total Rainfall[b]	Mean Max.	Mean Min.	Total Rainfall	Mean Max.	Mean Min.	Total Rainfall
November	75.2	55.6	2.95	74.1	50.5	2.55	77.9	52.5	2.75
December	72.7	52.0	4.49	69.1	43.0	1.78	71.7	53.3	1.40
January	73.7	50.2	1.32	[c]			68.6	41.8	2.56
February	74.6	48.8	0.99				78.4	54.1	1.41
March	76.0	51.0	1.78				76.2	50.8	0.17

[a] Expressed in degrees Fahrenheit.
[b] Expressed in inches.
[c] Recording instruments returned to manufacturer for repair.

somewhat less than 100 days was required to reach this level in the case of the two higher dosages.

Single applications of demeton at rates of 0.5 and 1 pound active ingredient per acre were made to the foliage of cabbage during the 1953–54 and 1954–55 growing seasons. Residue data are given in table 3. Excessive residues remained in the cabbage treated with 1 pound of actual demeton per acre 49 days before harvest. During the 1955–56 growing season three applications of demeton were made to the foliage of cabbage at 21-day intervals and at dosages of 0.25 and 0.5 pound per acre per application. The data presented in table 3 indicate that at both dosages detoxification to a safe level had occurred between 14 and 21 days after the last application.

Foliage applications of demeton to celery were made four times during the 1954–55 growing season, and five times during the 1955–56 growing season. The interval between applications varied slightly from the planned 21-day interval. Data on residues are presented in table 4 and indicate that at the dosage of 0.25 pound of demeton per acre per application a period of 21 days following the last application is required for degradation to or below the 0.75 p.p.m. level. Demeton residues in the 1954–55 celery treated with 0.5-pound dosage were in excess of 0.75 p.p.m. 28 days after the last of four applications. Residues in the 1955–56 celery treated five times each with 0.5 pound of demeton per acre were below the 0.75 p.p.m. level 21 days after the last application.

Temperature, rainfall, amount and intensity of wind, and sunlight, affect the rate of plant growth. These weather factors vary according to geographic location and the season of the year. The maximum and minimum temperatures for the period of the tests and rainfall are shown in table 5 to enable the reader to more closely evaluate the residue data, or make comparisons with data from other areas. Dates of application are presented in the tables with the data to which they are applicable.

During each of the three growing seasons that this study was in progress observations were made on aphid populations in the treated and untreated plots. Unfortunately aphid populations in all of the plots were so low that an evaluation of the effectiveness of the various dosages was impossible. However, very small populations of the cabbage aphid did develop in the untreated plots during the 1953–54 and 1954–55 growing seasons.

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Aphid Populations on Field Beans in Washington[1]

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Abstract

Aphid surveys were made in dry bean fields at Prosser, Wash., during the summers of 1954, 1955, and 1956. Seventeen species of aphids were added to the list of known species of the Yakima Valley. Yellow water-filled trap pan collections averaged over 90% *Myzus persicae* (Sulzer) with population peaks at the end of July in 1955, in midJuly in 1956. Collections of aphids from bean foliage showed higher proportions of *Macrosiphum granarium* (Kirby) and *Macrosiphum pisi* (Harris) and lower proportions of *Myzus persicae* than were found in the trap collections. More *M. persicae* alatae were found on the foliage of Red Mexican U. I. 34 beans than on the foliage of four other bean varieties. Aphids were unable to colonize on beans and usually only alate viviparae and some young nymphs were found on the foliage, apparently because the aphids are caught by the hooked hairs on the bean leaves and live only a short while.

In connection with a study of virus disease spread, a survey of the aphid populations in various dry or bush bean fields at the Irrigation Experiment Station in south central Washington's Yakima Valley was made during the summers of 1954, 1955, and 1956.

Aphids were collected from fields of six field bean varieties. In 1954 these varieties were: Red Mexican U. I. #34; in 1955, Pinto U. I. #72, Pinto U. I. #111, Red Mexican U. I. #34, Great Northern U. I. #16, and Early (Cali-

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fornia) Pink. In 1956, Red Mexican U. I. #34, Early Pink, and Columbia Pinto were grown.

Two methods of collection were used. Traps of the type developed by Moericke (1951) were utilized: 9-inch diameter, 1-quart pudding pans painted chrome yellow and filled with water. In addition, collections were made from the foliage of the bean plants in 1955 and 1956.

The trap pan collections were begun in midJune, when aphids began appearing in the fields, and were discontinued in midAugust, when virus symptoms had become well spread throughout the susceptible bean varieties. Various numbers of traps were used; they were placed within the fields on earth mounds to avoid their being hidden by the foliage of the plants about them.

Collections were usually made over a 24-hour period; *i.e.*, on one morning, the pans were placed in position in the fields and filled with water; on the following morning, the aphids attracted to the pans and drowned were removed. Some of the early summer collections, when aphids were few, were made over a 72-hour or 96-hour period. When aphid flights were heavy, it was found practical to strain out the aphids by pouring aphids and water through a fine mesh screen.

For the foliage collections, 25 leaflets or trifoliate leaves were taken at random from each of eight plots or sections of each bean variety planting on July 14, July 21, and August 4 of 1955, and July 16 and July 31 of 1956.

Easily-recognizable *Myzus persicae* (Sulzer) (green peach aphid) and *Macrosiphum granarium* (Kirby) (English grain aphid) were counted and discarded; the remainder were mounted on slides and sent to Miss M. A. Palmer of Colorado State University for determination. All aphids captured in the trap pans were alate viviparae. With the exceptions noted, most of the aphids collected from leaves were either alate viviparae or nymphs of the first or second instar.

NEW SPECIES FOR THE YAKIMA VALLEY.—Davis *et al.* (1954) reported collecting 139 species of aphids in the Yakima Valley. To this list, the present study has been able to add 17 new records. These are listed in table 1.

SEASONAL DISTRIBUTION.—The trap pan collections for 1955 and 1956 indicated seasonal patterns for some species.

In 1955, collections of all aphids were very slight and sporadic in June. Collections began to increase during the first half of July, and were maintained at a high level (50 aphids or more per pan) during the rest of July and the first half of August. This increase coincided with increases in *Myzus persicae*. This species constituted 85 to 98% of the aphids collected from pans during this period, with a peak number of 5,122 *M. persicae* in 12 traps on July 30. *Macrosiphum pisi* (Harris) (pea aphid) rose to 283 on July 22. The peak in collections of *Macrosiphum granarium* was 14, and of *Capitophorus braggii* (Gillette) (Oleaster thistle aphid), 89, on July 18; no more *M. granarium* were taken after August 3. It was noted, in the foliage collections, that roughly 10% of the *M. granarium* were parasitized on July 21, and nearly all of them, on August 4.

Collections of *Phorodon menthae* (Buckton) and *Aphis spiraecola* patch (with two exceptions) were confined to August, with peaks of 10 and 13, respectively, on August 11. Collections of *Hyalopterus atriplicis* (Linn.) (boat gall aphid) reached a peak of 123 on July 14, and *Capitophorus hippophaes* (Walker) had peaks of 30 on July 18, and 31 on August 5. Collections of other species of aphids were too small for any pattern to be suggested.

Table 1.—Additions to list of aphids of the Yakima Valley, reported by Davis *et al.* (1954).

SPECIES	WHEN AND HOW CAUGHT	
Aphis crataegifoliae Fitch	July 23, 1954	Trap
Aphis knowltoni Hottes & Frison	July 18, 1955	Trap
Aphis phaceliae Gillette & Palmer	July 5, 1954	Trap
Capitophorus carduinus (Walker)	Aug. 12, & 14, 1954	Trap
Capitophorus shepherdiae Gillette & Bragg	Aug. 4, 1955	Foliage
Drepanaphis granovskyi Smith & Knowlton	Aug. 3, 1955	Trap
Macrosiphum albifrons Essig	July 7, 1954	Trap
Macrosiphum creelii Davis	July 21, 1955	Foliage
Macrosiphum nigromaculosum Macdougall	July 16, 1956	Foliage
Myzocallis pulchellus Glendenning	July 20, 1956	Trap
Myzus monardae Davis	July 29, 1954	Trap
	Aug. 19, 1954	Trap
Pemphigus monophagus Maxson	June 22, 1956	Trap
Pemphigus populi-caulis Fitch	July 5, 1954	Trap
Rhopalosiphum padi (Linn.)	July 21, 1955	Foliage
Rhopalosiphum poae Gillette	July 16, 1956	Foliage
Therioaphis riehmi Börner	July 9, 1955	Trap
	July 21, 1955	Foliage
	July 22, 1955	Trap
	July 6, 1956	Trap
	July 17, 1956	Trap
Toxoptera muhlenbergiae Phillips & Davis	July 19, 1954	Trap
	July 29, 1954	Trap
	July 21, 1955	Foliage
	Aug. 4, 1955	Foliage

Collections of aphids in 1956 were few until June 26, climbed to a peak on July 17, fell off to less than one aphid per trap on August 3, and then began to climb again slowly. Once more, *M. persicae* constituted most of the collection, with a high of 2,239 (in 6 pans) on July 17. *Macrosiphum granarium* were few, and were lacking after July.

Collection peaks were reached for *Hyalopterus atriplicis* (51) and *Capitophorus hippophaes* (12) on July 13, for *Capitophorus braggii* (11) and *Macrosiphum pisi* (9) on July 20. Collections of *Aphis spiraecola* were made during midJuly and again during midAugust, being lacking in between. Other species of aphids were few.

A few of the trap pan collections have been tabulated to illustrate these seasonal variations (table 2).

PAN COLLECTIONS VERSUS LEAF COLLECTIONS.—Eastop (1955) has reported that the Moericke traps appear to be much more attractive to some species than to others. In the study dealt with here, certain contrasts in proportions between the pan collections and the leaf collections suggest similar differences. Some of the data pertinent to this point are listed in table 3. It should be kept in mind that while trap collections were for a single day, leaf collections may have involved accumulations of aphids over several days.

As indicated in the table, the trap pans attracted a greater proportion of *M. persicae* than were found on the leaves. On the other hand, a smaller proportion of *Macrosiphum granarium* and *Macrosiphum pisi* were found in

Table 2.—Numbers of the more abundant aphid species in a few Moericke trap collections.[a] Prosser, Washington.

DATE	*Aphis spiraecola*	*Capitophorus bragii*	*Capitophorus hippophaes*	*Hyalopterus atriplicis*	*Macrosiphum granarium*	*Macrosiphum pisi*	*Myzus persicae*	*Phorodon menthae*
1955								
July 1	0	0	1	1	1	5	0	0
July 8	0	2	2	2	0	33	28	0
July 14	1	12	18	123	6	35	226	0
July 18	0	89	30	7	14	72	660	0
July 22	0	9	16	2	3	283	3410	0
July 26	0	2	3	0	4	33	944	0
July 30	0	6	12	2	3	134	5122	0
Aug. 5	9	12	31	21	0	6	4500	3
Aug. 11	13	9	20	3	0	1	3175	10
Aug. 15	3	1	14	1	0	3	535	2
1956								
July 4	0	3	0	6	0	1	76	0
July 13	1	4	12	51	0	3	1851	0
July 17	6	3	8	23	3	8	2239	0
July 20	13	11	9	46	1	9	1599	3
July 27	0	1	10	5	1	0	185	0
Aug. 3	0	0	0	0	0	1	2	0
Aug. 14	49	1	1	14	0	1	27	0

[a] Twelve traps in 1955; 6 traps in 1956.

the traps than on the leaves. These trends, especially for *M. persicae* and *M. granarium*, were found throughout July of both years.

The causes of these variations are not known, though a number of possibilities can be suggested. There is, as indicated, a possible variation in attractiveness of the pans. The attractiveness of bean foliage may vary with aphid species. Conceivably, a variation might exist in the types of flight which would make some aphid species more susceptible than others to Moericke traps. The possibility that some aphids may be caught more readily than others by the epidermal hooks characteristic of bean leaves cannot be overlooked. Pea aphids in a trap pan have been observed to pull themselves out of the water, suggesting that the size and vigor of a species may be involved.

VARIATIONS ON DIFFERENT BEAN VARIETIES.—Most of the 1955 aphid collections were made in a 2-acre field of the five bean varieties mentioned earlier. These varieties were planted in a randomized block design—of eight replications. This arrangement, it was hoped, would negate the effect of proximity of some areas of the field to areas outside the field where various aphid species might be breeding.

The two July foliage collections for that year, listed in part in table 4, included a much greater proportion of *M. persicae* in the Red Mexican blocks than in those of the other four varieties. The collections of this aphid on July 14 constituted 36.8% of the Red Mexican collection of winged aphids, but only 13.3 to 18.5% of the collections from other varieties. Of the July 21 collections, *M. persicae* constituted 60.1% of the winged aphids from Red Mexicans, but only 42.7 to 46.8% from the other varieties.

Collections of other species of aphids were variable and

Table 3.—Comparisons of numbers of alate viviparae of three species of aphids caught in Moericke traps and collected from foliage.[a] Prosser, Washington.

SPECIES	JULY 14, 1955		JULY 21–22, 1955		JULY 16–17, 1956	
	Traps	Leaflets	Traps	Leaves	Traps	Leaves
Myzus persicae	226 (51.7)	140 (18.5)	3410 (91.4)	2257 (49.9)	2239 (97.8)	1455 (84.7)
Macrosiphum granarium	6 (1.4)	324 (42.8)	3 (0.1)	1066 (23.5)	3 (0.1)	131 (7.6)
Macrosiphum pisi	35 (8.0)	108 (14.3)	283 (7.6)	830 (18.3)	8 (0.3)	45 (2.6)
Other species	171	176	36	370	41	83
Total	438	758	3732	4523	2291	1714

[a] Figures in parenthesis are percentages of total collection for the particular day and method.

revealed no detectable apparent preference. The August 4 foliage collections and the trap pan collections, consisting of over 90% *M. persicae*, also revealed no readily detectable apparent preference.

APHID REPRODUCTION ON THE BEANS.—During the summer of 1955, perhaps the most noticeable attribute of the leaf collections was the apparent lack of any colonization on the beans by the aphids. Aphids found on the leaves were, with very rare exceptions, winged viviparae and nymphs of the first and second instars (mostly *M. granarium*), indicating that young aphids being produced on the bean foliage were not surviving past the second nymphal instar. One out of thousands was apterous, and perhaps came from weeds in the field.

The leaf collections of 1956 were similarly constituted, with one notable exception: the July 16 collection from the Red Mexican field included a considerable number of apterous adults and older (including alatoid) nymphs of *M. persicae*. Of the adults of this species taken in that collection, 263 (65.9%) were winged and 136 (34.1%) were apterous. Since the bean crop followed several years of alfalfa, the beans had a lush growth of foliage. This fact suggests a possible nutritional factor for the aphid or the plant which makes the plant more susceptible to colonization. However, the bean variety would be involved here too, since the Early Pink field had also been planted in alfalfa before 1956.

Observations of aphid survival on Red Mexican and Early Pink beans were also made in the greenhouse during virus transmission work in the winter and spring of

Table 4.—Numbers of the three most abundant species of aphids taken in collections from foliage in July 1955.[a] Prosser, Washington.

VARIETY	*Myzus persicae*		*Macrosiphum granarium*		*Macrosiphum pisi*	
	July 14	July 21	July 14	July 21	July 14	July 21
Pinto U. I. #78	27 (18.5)	386 (46.8)	75 (51.4)	197 (23.7)	11 (7.5)	175 (21.1)
Pinto U. I. #111	24 (13.3)	427 (44.8)	79 (43.9)	256 (26.6)	28 (15.5)	195 (20.2)
Red Mexican	43 (36.8)	473 (60.1)	46 (39.4)	171 (21.7)	10 (8.5)	93 (11.8)
Great Northern	27 (15.0)	564 (42.7)	57 (31.6)	203 (19.7)	35 (19.4)	177 (17.2)
Early Pink	19 (14.1)	404 (44.5)	67 (49.6)	239 (26.3)	24 (17.8)	190 (20.9)

[a] Each quantity is the number of winged aphids on 25 leaflets for July 14 and on 25 leaves for July 21. The figures in parenthesis are percentages of the total winged aphids for that collection and variety.

1956. Apterous adult aphids used in these tests were left on the test plants for 4 days following their transfer to such plant. At the end of this 4 day period, the aphids were examined.

Of 205 *Myzus persicae*, just 5 were still alive (of these, 3 were somewhat shriveled). A few live nymphs and several dead ones were present. The two healthy-appearing adults plus all the nymphs were found on Red Mexican test plants. Of 550 *Macrosiphum pisi*, 39 were alive, all somewhat shriveled, and two live nymphs (on Red Mexicans) and several dead ones were present. Of 200 *A. bakeri* (Cowen) (clover aphid), one weak one (on Red Mexican) was alive; no nymphs, dead or alive, were seen.

McKinney (1938) and more recently Johnson (1953) reported that the hooked epidermal hairs present on the bean foliage capture some aphids and as a consequence lower the chances of colonization of those aphids. "Aphid-hooking" was also observed during this study, though the extent to which it caused the apparent absence of colonization in most cases is not known.

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Effect of Molecular Configuration on Relative Toxicity to House Flies as Demonstrated with the Four *cis* Isomers of Allethrin[1]

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Abstract

l-Allethrolone *d-cis*-chrysanthemumic acid ester, the *d-l* ester, the *d-d* ester, and the *l-l* ester were, respectively, 0.33, 0.14, 1.8, and 0.056 as toxic to house flies (*Musca domestica* L.) as allethrin in space sprays applied by the Campbell turntable method. With the acid component of the molecule of the *l* form, the change from the *l* to the *d* form of the allethrolone component increased the toxicity of the ester by a factor of 2.4. With the allethrolone component of the *l* form, the change from the *l* to the *d* form of the acid component increased the toxicity of the ester by a factor of 5.8. With the common component of the *d* form, a similar change in the other component increased toxicity by factors 2.2 the two above. The effect of a change in each component simultaneously from the *l* to the *d* form was equal to the product of the separate effects. All the stereoisomers caused complete or nearly complete knockdown of flies at the concentrations used.

In 1953 a study was made of the relative toxicities of the four optically active isomers of *trans*-allethrin, prepared by chemists in the Department (LaForge *et al.* 1954), when applied in space sprays to house flies (*Musca domestica* L.) (Gersdorff & Mitlin 1953). It was shown that molecular configuration was of great importance in regard to the insecticidal action of these compounds, the most toxic, *d*-allethrolone *d-trans*-chrysanthemumic acid ester, being 150 times as toxic as the least toxic, the *l-l* isomer. The four optically active *cis* isomers were recently prepared by the same chemists (LaForge *et al.* 1956) and this paper reports the results of similar tests with them.

EXPERIMENTAL PROCEDURE.—In this paper the *cis* isomers in allethrin are designated as *l-d*, *d-l*, *d-d*, and *l-l*, the first symbol in the pair referring to the optical activity of the allethrolone component of the molecule and the second to that of the acid component. Thus, the first compound is *l*-allethrolone *d-cis*-chrysanthemumic acid ester. The samples were of high degree of purity.

A sample of allethrin, analyzing 93.4% by the hydrogenolysis method, was used as a standard of comparison. As an additional check a mixture of the four isomers in equal proportion was included.

The materials were dissolved in refined kerosene (Deobase) and diluted with the same solvent to form sprays for each at four or five concentrations selected according to preliminary toxicity tests.

Knockdown and mortality were determined by the Campbell turntable method on house flies of the National Association of Insecticide and Disinfectant Manufacturers' 1948 strain. All sprays were tested simultaneously and but once on each of six populations. Approximately 104 flies were used in each test. The average age of flies in each population was 2 to 3 days.

To evaluate relative toxicity and determine the precision of the estimates, the mortality data were subjected to probit analysis essentially as described by Finney (1952).

The mortality and knockdown data are summarized in table 1.

EVALUATION OF RELATIVE TOXICITY.—Provisional regression lines were fitted graphically to the mortality data. Since they showed no systematic departure from

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Table 1.—Mortality and knockdown of house flies caused by the *cis* stereoisomers of allethrin, their equiproportional mixture, and allethrin.[a]

Material	Concentration	Knockdown in 25 Minutes	Mortality in 1 Day
	Mg. per dl.	*Per cent*	*Per cent*
cis-Isomers:			
l-d	600.0	100	90.1
	400.0	100	79.3
	266.7	99.9	62.2
	177.8	99.9	37.4
	118.5	99.2	19.0
d-l	900.0	99.7	79.5
	600.0	100	59.0
	400.0	99.6	29.9
	266.7	97.1	16.4
d-d	100.0	100	87.0
	66.67	100	73.4
	44.44	100	51.6
	29.63	100	35.1
	19.75	98.6	18.3
l-l	2000	99.9	78.2
	1333	99.4	55.6
	888.9	96.7	25.4
	592.6	94.4	8.3
Mixture	300.0	100	92.9
	200.0	100	79.6
	133.3	100	58.5
	88.89	100	30.8
	59.26	99.4	12.5
Allethrin	214.1	100	93.4
	142.8	100	80.3
	95.17	100	68.3
	63.45	100	42.3
	42.30	99.9	21.7

[a] Six replications.

parallelism, a mean slope was calculated and equations were determined for lines of this slope. Expected probits were computed from these equations and the statistical procedure was followed. An analysis of chi-squares subsequent to generalization gave no evidence of conflict with the hypothesis of parallelism of lines. However, there was some heterogeneity between populations of flies, and a factor, 4.809, was required to allow for this in the calculation of variances.

The final equations showing the regression of mortality, expressed in probits, on concentration in milligrams per deciliter, expressed as logarithms, are given below. The standard error of the common regression coefficient is 0.110.

l-d Isomer	$Y=3.3418X-2.8522$
d-l Isomer	$Y=3.3418X-4.1053$
d-d Isomer	$Y=3.3418X-0.3982$
l-l Isomer	$Y=3.3418X-5.4020$
Mixture	$Y=3.3418X-1.9312$
Allethrin	$Y=3.3418X-1.2307$

From these equations the median lethal concentrations (LC-50's) were estimated and relative toxicities calculated as the inverse ratios of the relevant pairs of LC-50's. The estimations together with their standard errors are given in Table 2.

Table 2.—Relative toxicity of the *cis* stereoisomers of allethrin, their equiproportional mixture, and allethrin.

Material	LC-50	Toxicity Relative to Allethrin
	Mg. per dl.	
cis Isomers:		
l-d	223.7 ± 8.8	0.327 ±0.018
d-l	530.4 ±21.8	0.1380±0.0079
d-d	41.24± 1.62	1.775 ±0.099
l-l	1296 ±55	0.0564±0.0033
dl-cis Mixture:		
Found	118.6 ± 4.7	0.617 ±0.034
Expected	127.5	0.574
Allethrin	73.19± 2.9	1.0

Discussion.—Just as was demonstrated with the *trans* isomers and with the *cis* and *trans* factions (Gersdorff & Piquett 1957), the joint action of the *cis* isomers is shown by the results with the equiproportional mixture to be of the similar type. The toxicity of a mixture expected for that type of action may be calculated on the basis of equivalents according to the general equation given previously (Gersdorff & Mitlin 1952). Since the allethrin equivalents have been determined (table 2), they may be substituted in the equation as follows:

$$(0.25)(0.327)+(0.25)(0.138)+(0.25)(1.775)+(0.25)(0.056)=0.574.$$

The relative toxicity found for such a mixture, 0.617, is not significantly higher than that expected; therefore, it must be concluded that the four stereoisomers were acting similarly.

The two racemates of these *cis* isomers would be the equiproportional mixtures of the *l-d* and *d-l* esters and of the *d-d* and *l-l* esters. They have not been separated, since one of them does not crystallize out as does *α-dl-trans-allethrin*. However, their toxicities relative to allethrin may be calculated from the estimates for those of the optically active esters obtained in this study with the use of the general equation. These values are 0.233 for the first pair, which may be called *α-dl-cis-allethrin* in conformity with the corresponding *trans* pair, and 0.916 for the second, the *β* racemate. Since the two racemates constitute the *cis* fraction of allethrin and the relative toxicity of that fraction (0.546) has recently been determined by this method (Gersdorff & Piquett 1957), the proportion of the two may be estimated from the substituted equation, as follows:

$$p(0.233)+(1-p)(0.916)=0.546$$

in which p is the proportion of the α racemate and from which $p=0.542$ and $1-p=0.458$. Thus, by bioassay 54.2% of the *dl-cis* fraction consisted of the α racemate and 45.8% the β racemate. The *cis* fraction was found in a recent bioassay (Gersdorff & Piquett 1958) to be 29.7% of allethrin. If it is assumed that the ratio of α and β forms in the fraction is the same as in allethrin itself, which is likely with similar conditions of preparation, then the two racemates comprise 16.1 and 13.6% of allethrin, respectively.

The complete bioassay of the optically active constituents of allethrin may now be made. However, when es-

timations have been replicated, it seems best to use the means of the values found. Therefore, the proportions of the α and β racemates in the *trans* fraction have been recalculated with the use of these means, 0.286 for the toxicity relative to allethrin of α-*dl-trans* allethrin and 1.192 for the *trans* fraction, as cited later. The substituted equation is now

$$p(0.286)+(1-p)(1.685)=1.192$$

from which $p=0.352$ and $1-p=0.648$. Thus, 35.2% of the *dl-trans* fraction consisted of the α racemate and 64.8% the β racemate. Since the *trans* fraction was found by similar bioassay (Gersdorff & Piquett 1957) to be 70.3% of allethrin, the two racemates, on the same assumption regarding their ratio as with the *cis* racemates, comprise 24.7 and 45.6% of allethrin, respectively.

The complete assay is given below. From the relative toxicities found for the eight optically active isomers and their calculated percentages, the expected relative toxicities of the optically inactive racemates and fractions were determined from similar-action equations as above and are also given with their calculated percentages. For ready comparison with these expected values, the values found in previous work are given.

The value given for the *dl-cis* fraction is not to be confused with the estimation made on the 1950 preparation of this fraction (Gersdorff & Mitlin 1952), later found to have converted in part to the *trans* form. The agreement between the expected ratios and those actually found is excellent. The entomological method, then, shows allethrin when synthesized by the method of Schechter *et al.* (1949) to consist approximately of one-fourth α-*dl-trans*, one-half β-*dl-trans*, one-eighth α-*dl-cis*, and one-eighth β-*dl-cis* racemates. In fact, the toxicity expected for such a mixture is not significantly different from that of allethrin.

	Percentage in Allethrin	Toxicity Relative to Allethrin	
		Expected	Found
Optically active isomer:			
l-d trans	12.4	—	0.579[a]
d-l trans	12.4	—	0.138[a]
d-d trans	22.8	—	3.367[a]
l-l trans	22.8	—	0.0224[a]
l-d cis	8.0	—	0.327[b]
d-l cis	8.0	—	0.138[b]
d-d cis	6.8	—	1.775[b]
l-l cis	6.8	—	0.0565[b]
composite	100	1.023	1.0
Racemate:			
α-*dl-trans*	24.8	0.358	0.286[c]
β-*dl-trans*	45.6	1.694	1.685[d]
α-*dl-cis*	16.0	0.233	—
β-*dl-cis*	13.6	0.916	—
composite	100	1.023	1.0
Fraction:			
dl-trans	70.4	1.223	1.192[e]
dl-cis	29.6	0.547	0.546[e]
composite	100	1.023	1.0

[a] Gersdorff & Mitlin 1953.
[b] This study.
[c] Gersdorff *et al.* 1956.
[d] Gersdorff & Mitlin 1952.
[e] Gersdorff & Piquett 1957.

Crombie & Harper (1954) deduced the configurations of the optically active chrysanthemumic acids, and Elliott (1954) discussed them as well as those of some esters synthesized from these acids. Elliott also discussed the association of insecticidal activity with stereochemical conformation. With the present completion of the estimation of all eight stereoisomers by one method, it is apparent that there is a much greater disparity between the most and the least toxic esters from the *trans* form (ratio of 150 to 1) than from the *cis* form (ratio of 31 to 1). However, as will be indicated later, definite correlation of insecticidal activity with conformation is not so simple with compounds that are very difficult to prepare absolutely free of small amounts of isomers, some of which differ so widely in toxicity. It is possible that some of the *cis* samples are contaminated with the *trans* form because of partial conversion, and with some preparations this could be serious with regard to toxicity.

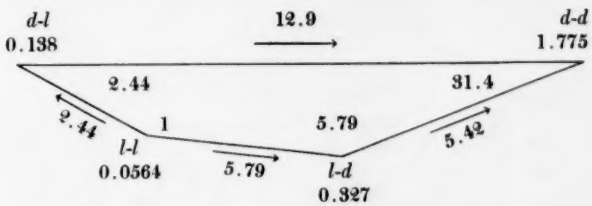
The relative toxicities of the *cis* stereoisomers as found herein do not fall into a regular pattern, as do the *trans* compounds. If the proper LC-50's (or ratios to allethrin) given in table 2 are compared, the following ratios of toxicity will be obtained:

$$\left.\begin{matrix} d\text{-}d/d\text{-}l=12.86 \\ l\text{-}d/l\text{-}l=5.79 \end{matrix}\right\} 12.86/5.79=2.22$$

$$\left.\begin{matrix} d\text{-}d/l\text{-}d=5.42 \\ d\text{-}l/l\text{-}l=2.44 \end{matrix}\right\} 5.42/2.44=2.22$$

$$d\text{-}d/l\text{-}l=31.4$$

Thus, the toxicity of the ester is higher when either the acid or the allethrolone component of the molecule is dextrorotatory rather than levorotatory with the other component of one form; the ratio of toxicity is about twice as great when the common component is dextro as when it is levo. With the *trans* arrangement in the acid component, the ratios in each pair are the same—about 6 when the change is in the allethrolone component and about 25 when it is in the acid. The pattern for these samples of the *cis* isomers, then, may be illustrated by an irregular quadrilateral as given below, not by a simple rectangle as with the *trans* isomers (Gersdorff & Mitlin 1953).



Each side of the diagram represents the relative toxic effect accompanying a change in configuration in one component of the molecule, the arrows pointing in the direction of increasing effect. The effect of a simultaneous change in each component from the *l* to the *d* form is equal to the product of the separate effects. Study of the diagram or the ratios shows quickly that if a more nearly true estimation was 0.45 of that found for either the *l-l* or the *d-d* isomer, symmetry would be obtained and a simple conclusion with regard to the effects of the *cis* arrange-

ment would be possible. However, the samples were of high degree of purity and there are no indications that either of these altered ratios is likely. In fact, such a change in the estimate for the *d-d* isomer could not be true, for then the hypothetical toxicity of the β racemate would be 0.427 (instead of 0.916) and both racemates would be less toxic than the *cis* fraction itself (0.546), and also the toxicity of an equiproportional mixture (0.330) would be almost half that found (0.617). Because of the low toxicity of the *l-l* isomer, the effect of such a change in its estimate would not be great enough to demonstrate impossibility in similar comparisons, the figures being 0.900 and 0.566, both within the confidence limits of the values found. On the other hand, relatively small amounts of certain isomers would be required to cause such a difference in the estimation for the *l-l* isomer. For example, admixture of only 1% of the *d-d trans* ester or 2% of the *d-d cis* ester would be necessary to raise the evaluation of an ester with the ratio of toxicity of 0.0224 (relative toxicity of *l-l trans* ester) to that found for the *l-l cis* ester.

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Preliminary Studies of Entomogenous Fungi for the Control of Potato-Infesting Aphids in Maine[1]

W. A. Shands,[2] C. G. Thompson,[2] G. W. Simpson,[3] and H. E. Wave[2]

Abstract

In one out of four tests in 1955 at Presque Isle, Maine, an introduced fungus *Acrostalagmus*, probably *aphidium* Oud., was recovered from dead specimens of the potato aphid, *Macrosiphum solanifolii* (Ashm.), in plots of potatoes 3 weeks after the plants with a light aphid infestation had received a single spray application of laboratory-cultured spores. In two other field tests no similar recovery was made following application of spores of a locally-occurring, undescribed species of *Empusa* to *Rosa* spp. infested with *M. solanifolii*.

Entomogenous fungi have frequently exhibited a significant degree of aphid control in potato fields in northeastern Maine. This paper summarizes observations over an extended period in this area, and also reports preliminary tests conducted near Presque Isle in 1955 to determine whether two of these fungi—an apparently undescribed species of *Empusa* occurring locally, and *Acrostalagmus*, probably *aphidium* Oud., not formerly recorded from the Northeast—could be established by disseminating laboratory-cultured spores.

The Fungus Disease-Aphid Complex.—All four aphid species commonly infesting potatoes in Maine have been found to be attacked by as many species of fungi, with some specificity evident. The potato aphid (*Macrosiphum solanifolii* (Ashm.)) appears to be most susceptible to infection and the buckthorn aphid (*Aphis abbreviata* Patch) the least. The green peach aphid (*Myzus persicae* (Sulz.)) and the foxglove aphid (*Myzus solani* (Kltb.)) are intermediate in susceptibility.

Based on the frequency of occurrence of dead aphids infected with entomogenous fungi[4] in the many collections made since 1941, *Empusa* (*Entomophthora*) *aphidis* (Hoffman) has been the predominating species, followed by *E.* (*E.*) *sphaerosperma* Fres. The undescribed *Empusa* proved of unusually great importance in 1954 and 1955. Harris (1948) reported the isolation of *Entomopthora coronata* (Cost.) Kev. from a collection of dead diseased green peach, potato, and foxglove aphids from which he also recovered *aphidis*. He was able to culture *coronata* and to infect healthy green peach aphids with it in the greenhouse. This species has not been recovered from any of the dead diseased aphids collected since 1947.

Nearly 70 years ago Thaxter (1888) mentioned several species of *Empusa* as affecting aphids, with some of the records from Maine. Patch (1907) found that *aphidis* caused heavy mortality of the potato aphid in fields of potatoes near Houlton in 1907, and she reported that fungi sometimes practically exterminated this aphid over wide areas.

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[4] Some of the determinations of fungi were made by Vera K. Charles, John A. Stevenson, and Flora G. Pollack, of the U. S. Department of Agriculture.

Table 1.—Description of field tests to establish *Empusa* and *Acrostalagmus* fungi as parasites of potato-infesting aphids.

Plant	Test[a] No.	Time of Application: Date (1955)	Time (P.M.)	Millions of Spores per cc.	Post-Application Observations
			Empusa		
Rugose rose	1	June 13	6:30–7:00	0.91	June 20
Swamp rose	2	June 13	7:00–7:30	.91	June 20
			Acrostalagmus		
Potato	3	June 22	4:50–5:15	4.14	June 28
	4	July 20	5:30–6:15	.64	July 24, 28
	5	July 28	7:00–7:30	.53	Aug. 1, 5, 12, 22
	6	Aug. 5	6:45–7:15	.53	Aug. 9, 12, 26

[a] Tests 4 and 5 were on the same plots.

Table 2.—Weather conditions during the first week following application of *Acrostalagmus* spores in field tests at Presque Isle, Maine, in 1955.

Test	Temperature (°F.): Range	Mean	Precipitation: Days	Inches
1, 2	52.6–75.9	64.2	3	0.99
3	53.4–75.3	64.4	5	.36
4	52.7–78.1	65.4	4	1.49
5	51.7–80.3	66.0	1	.07
6	52.4–77.4	64.9	5	1.19

Our records show that, although not always of apparent economic importance, fungus diseases have frequently exerted a significant degree of aphid control in fields of potatoes in northeastern Maine. They have operated to some extent in the potato fields each year since 1941. Epizootics have usually occurred about mid-August, or when the aphid population was near its peak on potatoes. Even during the years in which the beneficial effect of the fungi has been less spectacular, the overall control may have been even greater because early-season effectiveness reduces the general level of aphid abundance throughout the season (Shands *et al.* 1955).

FIELD TESTS.—*Acrostalagmus* fungus for producing the spores used in the field tests was initially recovered early in 1955 from a collection of dead specimens of the corn leaf aphid (*Rhopalosiphum maidis* (Fitch)) from Hawaii; the *Empusa* was from Maine. Cultures of the spore material were grown at Beltsville, Md., by three procedures. Two of them were alike except for treatment just before airmail shipment to Maine. Cooked slices of potato tuber about ⅜ inch thick inoculated with the spores were held in Petri dishes at a constant temperature of 76° F. for 10 days or until a heavy crop of spores was ready to harvest. The moist potato-slice cultures were used as source material for the first two of the six field tests, but to facilitate shipment for the next two tests the mature cultures of *Acrostalagmus* were stored at room temperature until dry. The third procedure, used for the last two tests, consisted in propagating the fungus on potato-infusion agar supplemented by 1% of dry yeast.

Upon their receipt at Presque Isle, the mature cultures were stored at 42° F. until the sprays were prepared for field applications. To prepare the spray suspension of spores, the culture material was mixed with rain water and aliquots were placed in separate containers. Definite rates of mixtures containing a uniform spore concentration could thus be applied to the plot replicates regardless of size. From 1½ to 2 hours after the cultures had been taken from storage field application of the spray was started.

One of the six field tests was made on rugose rose (*Rosa rugosa* Thunb.), two on swamp rose (*Rosa palustris* Marsh.), and three on Green Mountain potatoes. Natural colonies of the potato aphid were caged 17 days before the spray was applied on rugose rose and in the first test on swamp rose to insure large populations. Only unprotected, natural infestations were used in the other test on swamp rose and in the three tests on potatoes. The cages were removed to increase spray penetration of the dense rose foliage.

Application to the roses was made with a small hand-plunger atomizer; thorough coverage was obtained by directing the spray from several angles. Potatoes were treated with a small precision-type machine that delivered the spray at a constant pressure of 52 pounds per square inch from a single nozzle on the end of a hand-operated boom. This machine was operated to deliver 125 gallons per acre. Its long, upturned boom made possible delivery of much of the spray to the undersides of the leaves as the operator walked through the plots. Each plot of potatoes consisted of four 34-inch rows 15 feet long and contained 60 plants. There were four plot replications for each of the tests on potatoes. All plots were sprayed weekly throughout the summer with a water suspension of yellow cuprous oxide at the rate of 3 pounds per acre for the control of late blight (*Phytophthora infestans* (Mont.) DBy.).

Table 1 contains descriptive data for each test, including dates of applications and subsequent observations for dead aphids. Determinations of fungi were made for all dead aphids collected at each post-application observation.

Although many fungus-killed aphids were collected from the plants, none killed by *Empusa* sp. were found in tests 1 and 2 and *Acrostalagmus*-infected specimens were recovered only from potatoes in test 6, and then not until August 26, or 3 weeks after the spore application. The potato aphid was the only species from which the fungus was recovered. Of the few disease-killed specimens collected, about 60% were infected with *A. aphidium*, and the remaining ones with *Empusa* sp., *E. aphidis*, or *E. sphaerosperma*. Specimens infected with *A. aphidium* were taken from three of the four plots to which spores had been applied. The finding of two similarly infected potato aphids in one of the three adjacent check plots that had been sprayed with water only on August 5 indicated some spread of the fungus.

Gilliatt (1925), Dustan (1927), and McLeod (1956) have made valuable contributions to the biology of *Empusa*, but little is known of the biology of the different species under natural conditions. Therefore, we cannot give a critical appraisal of the environmental factors that affected the chances of the fungi becoming established during our tests. It appears, however, that weather conditions (table 2) were fairly favorable in four of the tests. Despite the rainfall following the application in test 4, the humidity was rather low because of fairly high daytime temperatures. It was also fairly low following the ap-

plication in test 5 because of little rainfall and relatively high daytime temperatures.

These tests indicate that large populations of the potato aphid are not necessary for establishment of *Acrostalagmus* in the field. Although fairly high populations of the aphid were on the roses, they were unusually low on the potatoes. At the time of spore application in the plots where *A. aphidium* became established, there was less than one potato aphid per three leaves, as determined by the method of Shands & Simpson (1953).

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The Determination of Some Insecticidal Residues in Soils by Bioassay[1]

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ABSTRACT

Soil samples having received heavy applications of DDT, toxaphene, dieldrin and BHC were bioassayed for residues. Extracts were obtained from 0 to 3 and 3 to 6 inch levels by use of a 4:1 solution of hexane and acetone. A photomigration technique, employing the use of mosquito larvae, was followed for making the analyses. A comparison is made of the amounts of residues of the insecticides recovered.

Harmful residues, due to numerous applications of insecticides to plants and animals, have been investigated for several years. Headen (1908), working in Colorado, found that sufficient arsenic had accumulated in soil following applications of lead arsenate to cause injury to fruit trees. Fleming (1950) stated that the residual effectiveness of DDT appeared to be as permanent as that of lead arsenate when applied to soil for control of the Japanese beetle larvae and observed effective control of the pest for 5 years following the application of 25 pounds of DDT per acre.

Chisholm *et al.* (1950) stated that most of an accumulation of DDT in orchards occurred within 3.0 inches of the surface of the soil. Lange & Carlson (1955) recovered 74% more dieldrin from the upper 2½ inches of treated soils than from the 2½ to 5.0 inch level following various tillage methods. DDT used as a seed treatment on beans and carrots delayed germination but did not impair growth, Morrison *et al.* (1945a, 1945b). An application of 100 pounds of BHC (30 pounds of gamma isomer) per acre to the soil greatly reduced germination and early growth of peanuts, corn, cotton and soybeans, Grayson & Poos (1947).

The objective in the present study was to determine the amount of insecticidal residues in soils by some means of biological assay. Bioassay would ascertain if residues toxic to the test animals used remain in the soils treated with insecticides. Sun & Sun (1952) have pointed out that a chemical analysis is based upon the reaction of a reagent to a certain structure of a compound and does not necessarily indicate the presence of a toxic substance. The com-

[1] Part of a thesis submitted by Robert S. Berger to Texas A. & M. College in partial fulfillment of the requirements for a degree of Master of Science.

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Table 1.—Residue determinations by bioassay of insecticides applied to soils in 1955 and 1956 at College Station, Texas.

INSECTICIDE	POUNDS OF TOXICANT PER ACRE	RESIDUES DETERMINED							
		0 to 3 Inch Level			3 to 6 Inch Level			Total	
		Lbs./A	Per Cent	P.P.M.	Lbs./A	Per Cent	P.P.M.	Lbs./A	Per Cent
DDT	66.5	4.9	7.4	5.6	6.6	9.9	7.4	11.5	17.3
Toxaphene	125.1	7.8	6.2	9.2	13.3	10.6	14.9	21.1	16.9
Dieldrin	11.2	1.3	11.6	1.5	1.2	10.7	2.9	2.5	22.3
BHC[a]	66.5	1.5	2.3	1.7	1.1	1.6	1.3	2.6	3.9

[a] Gamma isomer 25%; other isomers 75%.

Table 2.—Percentages of total insecticides present in the 0 to 3 inch and 3 to 6 inch levels of treated soil, based upon bioassay tests.

Soil Level (Inches)	Replicate No. of Sample				Average
	1	2	3	4	
			DDT		
0–3	7.2	7.7	7.8	6.9	7.4
3–6	10.7	13.4	7.5	8.1	9.9
			Toxaphene		
0–3	6.7	6.6	6.1	5.4	6.2*
3–6	12.1	11.5	9.5	9.7	10.7*
			Dieldrin		
0–3	18.1	7.0	16.6	5.4	11.8
3–6	4.8	13.6	10.7	12.0	10.3
			BHC[a]		
0–3	2.7	2.1	2.5	1.5	2.2
3–6	2.3	1.5	1.6	1.4	1.7

[a] Gamma isomer 25%; other isomers 75%.
* Significant at the 1% level.

bination of results obtained by chemical analysis and bioassay probably would give a better indication of the nature of the residues determined.

MATERIALS AND METHODS.—Soil samples were obtained from a field experiment which consisted of 20 one-ninth acre plots arranged in randomized blocks. Treatments included heavy applications of DDT, dieldrin, toxaphene and BHC and were made during 1955 and 1956, table 1. All insecticide treatments and the untreated check were replicated four times. A soil sampling tool described by Johnson & Caskey (1952), slightly modified for use in this study, was employed to remove cylindrical cores of soil 2 inches in diameter from the 0 to 3 inch and 3 to 6 inch levels. Insecticidal residues were removed from the soil samples by stripping with a 4:1 mixture of hexane and acetone. Soil extracts in acetone were used to prepare test suspensions.

A bioassay method employing the use of second instar larvae of the yellow fever mosquito, *Aedes aegypti* (L.), as described by Burchfield & Hartzell (1955), was followed for the determination of insecticidal residues in soil samples. This technique was based upon the negative phototactic response of the larvae. Each photomigration test was repeated until 70 to 80% of the mosquito larvae were inactivated. The percentage of the larvae inactivated at certain time intervals was plotted in probits against time. The time required for inactivation of 50% of the larvae (T50) was determined by interpolation. Standard time-effect curves of each insecticide were constructed for solutions containing various amounts of toxicant. These curves were obtained from readings made in groups of four replicated four times. The curves representing the reaction of mosquito larvae to solution containing unknown amounts of toxicant were made in the same manner. Blank extracts of untreated soil were not run since all treated samples contained sufficient insecticide residues to give more rapid inactivation of the mosquito larvae than the standards of the least amounts of the insecticides. Eight suspensions (4 standards and 4 unknowns) were tested at the same time using lots of mosquito larvae taken from the same rearing batch. The averages of the T50 values of the four tests conducted for each insecticide were used in making calculations.

RESULTS.—A summary of the insecticidal residues determined in the soils, expressed in pounds per acre, is presented in table 1. Of the DDT and toxaphene applied to the field plots approximately 17% was recovered in the residue analysis. Dieldrin appeared to be more stable; 22.3% of the total insecticide applied was recovered. Less than 4% of the BHC applied to the field plots was recovered.

A comparison of the amounts of insecticides determined in the soil samples, table 2, shows that significantly more toxaphene was recovered from the 3 to 6 inch level of the treated soils. No significant difference was found between the quantities of insecticides determined in the 0 to 3 inch and 3 to 6 inch levels of BHC-, DDT- and dieldrin-treated soils.

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Newer Acaricides and Insecticides in the Control of Ectoparasites of Poultry[1]

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ABSTRACT

The northern fowl mite (*Ornithonyssus sylviarum* (C. & F.)) presents an economic problem of increasing importance in Georgia, nearby states, and probably in other parts of the United States. Increasing incidence is here reported from 18 states while only Washington reported a decrease. Preliminary screening tests were conducted with seven chemicals. Of these 5% Sevin dust, 1.5% dieldrin dust, 25% S-MP powder, and 25% PS-256 powder were found ineffective in control. Dow ET-57, Sulphenone, and Trithion showed promise. Field testing showed that 10% Sulphenone dust reduced the population but did not eradicate the mite. In two field experiments 5% Dow ET-57 dust brought about complete eradication for 8 or more weeks after treatment. Dow ET-57 was also effective in eradication of four species of lice: *Eomenacanthus stramineus* (Nitz.), *Menopon gallinae* (L.), *Lipeurus caponis* (L.) and *Goniocotes gallinae* (DeG.) from 195 hens in one of the field trials. Similar results were achieved on the northern fowl mite using 5% Trithion dust. Five per cent malathion dust eradicated an infestation of the sticktight flea, *Echidnophaga gallinacea* (Westw.). An experiment on hatchability showed no effects upon 90 birds treated with Dow ET-57 as compared with 90 untreated controls. No unfavorable taste effects upon eggs or toxicity symptoms in birds treated with Dow ET-57 or Trithion were observed in these tests.

The northern fowl mite, *Ornithonyssus sylviarum* (C. & F.), has become a serious economic pest in Georgia where poultrymen have experienced inadequate control with generally accepted acaricides. An increasing problem was indicated also in 17 other states in replies to an inquiry on northern fowl mite incidence sent to Heads of Entomology or in some cases to Poultry Departments in the 48 states and Canada. Only Washington State indicated a decreasing problem. Three states bordering on Georgia, —Alabama, North Carolina and South Carolina—reported increasing incidence. Other states which reported an increase were Indiana, Iowa, Kentucky, Ohio, Oregon, Michigan, New Hampshire, New Jersey, New York, North Dakota, South Dakota, Texas, Vermont, and Wisconsin. Thirty-nine states and Canada indicated that the mite was present while nine states reported no experience with the problem. Although data thus obtained may have limitations, they give some idea of the distribution of *O. sylviarum* at the present time.

The increasing size of commercial poultry flocks requires newer methods of mass treatment for control of external parasites. Individual dusting or dipping of birds, even though effective, is impractical with large flocks. Mass flock treatment is carried out either by spraying or dusting. Since the average farmer usually does not have the necessary spray equipment, only dusting methods were considered for studies of chemical treatments. Dust may be applied easily by hand for floor birds or with a simple puff duster under caged birds.

Malathion has been highly recommended for control of this pest by Furman & Coates (1957), and others. However, a testing program begun in 1955 with 4% and 5% malathion dusts, carried out according to manufacturer's directions, failed to eradicate the mite on the College Experiment Station Poultry Farm (Reid *et al.* 1956). Edgar (1956) and Schmittle (1957) have also indicated its ineffectiveness in control of this pest in Alabama and Michigan. Since numerous synthetic acaricides have been developed for plant and animal work in recent years a testing program was initiated to select a more effective chemical. Control of *O. sylviarum* was studied by screening experiments, field testing and some special toxicity studies. During the course of the field testing an opportunity appeared to apply new compounds for control of lice and the sticktight flea.

MATERIALS AND METHODS.—Plastic catsup dispensers were used to apply dusts to individual birds in screening experiments. In field experiments with floor birds dust was applied by hand to nests, litter and roosting areas. Since information on toxicity was limited on several of the chemicals, rubber gloves and a face mask were used in making the applications.

Except for the commercial flock treated for sticktight fleas all infestations treated were due to natural outbreaks on College Experiment Station farms. Each group of infested birds was examined for mites or lice and scored before and after treatment. The scoring system used was a combination of the methods of Moore & Schwardt (1954) and Edgar, *et al.* (1949). A numerical score was given to each bird and the average pen scores computed according to the following scale:

Number Rating	*Degree of Infestation*
0	No mites seen
1	Less than 10 mites seen
2	Light infestation
3	Medium infestation
4	Heavy infestation
5	Very heavy infestation

A single operator scored all the birds in any one experiment. The numerical score was unimportant after treatment since eradication was the rigid requirement set up

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for any acaricide or insecticide. Eradication was not considered achieved unless all birds were negative (a score of 0.0) for ectoparasites for at least 8 weeks after treatment. The 30-day period accepted by Furman & Coates (1957) was considered too short for the eradication standard set up in the present studies.

In screening experiments seven different chemicals were used. These were: 5% Dow ET-57 dust (sampled as Dow ET-14 and also know as Korlan); 5% Sevin dust (N-methyl-1-naphthyl carbamate); 1.5% dieldrin dust; 25% S-MP powder (dimethyl phthalate); 25% PS-256 powder (2-ethylhexanediol-1,3); 10% Sulphenone dust; and 5% Trithion dust. The Dow ET-57, Sulphenone and Trithion together with 5% malathion dust were used in field experimentation.

RESULTS WITH *O. sylviarum*.—*Experiment 1.*—In September, 1956, individual birds were treated separately with each of the first five chemicals to be screened. After hens had been scored for mite infestation, each bird was given approximately 4 grams (one puff) from the dispenser and the chemical was then rubbed around the vent area. Treated birds were placed in individual houses to preclude contamination by other chemicals. Birds were examined periodically for 20 days. Only 5% Dow ET-57 gave complete eradication considered necessary for effective control. Sevin reduced the mite population but did not eradicate it. Dieldrin, S-MP and PS-256 had no apparent effect on the mite population.

Experiment 2.—In January, 1957, similar screening tests were made with 10% Sulphenone and 5% Trithion. Both of these chemicals brought about complete eradication and the birds remained negative for 55 days. These screening tests indicated that Dow ET-57, Sulphenone and Trithion were effective enough to warrant field testing.

Experiment 3.—Field testing experiments were started in November with 5% Dow ET-57. Four pens of heavily infested cockerels were brought in from the range and housed in a breeder house. Pen 1 had an average score for mites of 5.0 while pen 2 had 4.9, pen three 0.9 and pen four 0.2. Scores below 1.0 indicate inclusion of negative birds. Mass treatment of litter could not be used at this time due to limited quantities of available chemical. Individual treatment consisted of approximately 8 grams from a dust gun for each bird. Two days after treatment rating by pens was 0.6 for pen one, 1.6 for pen two, 0.2 for pen three and 0.0 for pen four. On the third day the population was further reduced to 0.2, 0.9, 0.0 and 0.08. Some increase in population was apparent on the 18th day when the score was 1.0 for pen one, and 1.4 for pen two. During this treatment period live mites were observed crawling on the framework of the house. A few remaining birds in the very heavily infested range shelters from which these cockerels had come also were treated. No consistent decrease in mite population occurred. It appeared from this experiment that Dow ET-57 had definite acaricidal properties but that eradication would depend upon using more of the chemical for litter and pen treatments.

Experiment 4.—Birds heavily infested with mites were moved in from the range into colony-type isolation houses with 208 square feet of floor space. New pine shavings to a depth of 4 inches were used as litter. Twenty-three hens and three cockerels had an average initial mite rating of 3.2 per bird. Five per cent Dow ET-57 dust at the rate of 1 pound per 40 square feet was applied by hand to litter and nests. Subsequent ratings were all 0.0 or negative for mites 1, 2, 4, 7, 8, 10 and 11 weeks after application of the chemical.

Experiment 5.—A natural infestation of *O. sylviarum* occurred in one of the breeder houses containing 30 individual pens, each pen measuring 8′×10′ (80 square feet). The pens, each of which contained 13 hens and one cock, were separated by a 4-foot cement aisle with 15 pens on both sides. Fifteen pens on the north side of the house were used in treatment studies. Seven individual birds from each pen were selected at random and rated for infestation. During the month of January the average rating per bird was 2.6 for mites in 7 of the 15 pens whereas no mites were found in the other eight pens. Dow ET-57 dust was applied at the rate of 1 pound per 20 square feet. All subsequent ratings were negative for a period of 8 weeks. Fifteen pens on the south side were used as untreated controls. Mite populations in these pens remained unchanged during the treatment period.

Experiment 6.—Ten per cent Sulphenone dust was used to treat 18 hens and one cock in another colony-type isolation house during the month of April. The average initial mite rating was 3.8. Dust was applied at the rate of 1 pound per 20 square feet. Mites were still present on several birds after 28 days. Since eradication was not achieved no further testing of this compound was carried out.

Experiment 7.—Five per cent Trithion dust was applied in a similar experiment to 20 hens during the month of May. The initial average rating for mites was 3.5 per bird. All subsequent ratings over a period of 9 weeks were negative. This compound thus shows potential effectiveness in the control of poultry ectoparasites.

LOUSE CONTROL.—In Experiment 5 all birds infested with mites also harbored one or more species of lice. Lice were present on all the hens in all the pens including those which were negative for mites. The species of lice present were: the chicken body louse, *Eomenacanthus stramineus* (Nitz.), the shaft louse, *Menopon gallinae* (L.), the wing louse, *Lipeurus caponis* (L.), and the fluff louse, *Goniocotes gallinae* (DeG.). The average initial rating for lice was 3.2. Dow ET-57 gave complete control, and subsequent ratings were zero in all 15 pens for 8 weeks. Louse populations in the untreated control pens remained unchanged during the treatment period. It would thus appear that Dow ET-57 is effective in control of lice as well as mites.

CONTROL OF THE STICKTIGHT FLEA.— A commercial egg flock consisting of 1,500 pullets which were just coming into production had an infestation of the sticktight flea, *Echidnophaga gallinacea* (Westw.). They had been housed in confinement but were occasionally let out to range. Scoring did not appear practical but the average infestation would be rated as light. No reports were available on the use of malathion in control of the sticktight flea. A 5% dust was applied by hand over the entire house (3,840 square feet) at the rate of approximately 1 pound per 75 square feet. After 2 weeks live fleas were still present on some of the birds. Some re-infestation might have occurred when the owner let the pullets out to range. A second application of 1 pound per 20 square feet

made at this time brought about complete eradication. Birds were confined to the house after the second treatment. No further fleas were found on subsequent examinations made at weekly intervals for 8 weeks. Malathion dust would thus appear to be an effective method of control of this pest.

EFFECTS OF CHEMICALS ON HATCHABILITY AND TASTE.—From its effectiveness in eradication of both lice and mites in experiments 4 and 5 Dow ET-57 appeared to be a promising chemical for control of poultry ectoparasites. Expanded field studies, however, are not warranted until further toxicity data have been released. An experiment was set up to test possible effects of Dow ET-57 on hatchability in conjunction with other studies in the Poultry Department.

One hundred eighty White Leghorn hens which had first been artificially inseminated were divided into two groups. The first group of 90 birds was well covered with 5% Dow ET-57 dust using approximately 12 grams (three puffs) directed into the vent area from under their cages. The second group of 90 birds was used as controls. Eggs were set from both groups of hens in two separate hatches. Eighteen days after setting there were 112 infertile eggs from the treated group and 121 from the controls. On the 22nd day treated birds produced 256 chicks as compared with 288 from untreated hens. There were 58 dead embryos in each group. No significant difference was found in hatchability between treated and untreated hens, indicating no toxic effects from this chemical.

During the course of experiments 4 and 5, with Dow ET-57, as well as in experiment 7, with Trithion, eggs were periodically submitted to a taste panel to determine the effects of Dow ET-57 and Trithion on taste. Eggs were sent to four households with a total of 12 people. The request that any flavor differences be reported was made. No off-flavor was detected by the panel.

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Greenhouse Technique for Evaluating Sweetclover Weevils' Preference for Seedling Sweetclover Plants[1]

R. V. CONNIN, H. J. GORZ, and C. O. GARDNER[2]

ABSTRACT

A method for evaluating preference of adult sweetclover weevils (*sitona cylindricollis* Fahr.) for seedling sweetclover plants is discussed. Seedling plants of a homozygous weevil-susceptible selection of *Melilotus alba* were subjected to the feeding of various numbers of adult weevils. Replicated tests were made with 1, 2, 4, and 6 weevils per plant. Statistical analyses run on the individual plant data showed significant differences between rows of plants. When assumed varieties were randomized so that each appeared the same number of times in each row, no significant differences were noted. Five varieties per flat replicated in two 5×5 Latin squares infested with two weevils per plant produced the most reliable results. The use of a homozygous susceptible standard check will provide, for flat to flat, and year to year, comparisons of materials tested.

In recent years the sweetclover weevil (*Sitona cylindricollis* Fahr.) has been responsible for a material reduction in the acreage of sweetclover in the United States. The feeding of the overwintered adults on emerging seedlings has made it difficult to establish and maintain stands. There is general agreement among investigators that a new planting can withstand fairly large numbers of weevils if it can be grown beyond the seedling stage. An exception to this generality occurs in areas such as the Red River Valley of North Dakota, Minnesota, and Canada, where sweetclover is grown as part of a rotation program for the production of sugar beets. In this area many first-year plants 18 to 24 inches in height are completely defoliated and killed by the feeding of adults that have migrated from second-year stands that were plowed down for green manure.

Allen (1955), Allen & Kelleher (1954), and Haws & Holdaway (1954) have demonstrated that the sweetclover weevil can be controlled with any of several insecticides. Haws (1954) and Wilson *et al.* (1956) found in greenhouse and field studies that Common Yellow and Common White sustained less injury by adult weevils than other varieties. It was therefore suggested that control of this weevil might be accomplished by the selection of plants that are resistant to the adult and/or larval stages. Since the adult is easier to study, it was chosen for initial resistance studies. A greenhouse technique for evaluating pref-

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[2] Respectively, Entomology Research Division and Crops Research Division, Agr. Res. Serv., U.S.D.A., and Nebraska Agricultural Experiment Station.

erence for seedling plants by adult weevils and a statistical analysis of the technique are presented herein.

MATERIALS AND METHODS.—Weevils for use in the greenhouse studies were collected in September 1955 in the Red River Valley near Crookston, Minnesota. After they had been separated from debris and other insects, the weevils were divided into lots of 500, placed in quart ice-cream containers with strips of moistened paper toweling, and stored at 34° F. until used. Tap water sprayed into the containers at weekly intervals provided adequate moisture. Approximately 5% mortality occurred among weevils stored in this manner.

Adult sweetclover weevils are very active and capable of walking or flying considerable distances. Thus in a greenhouse program for evaluation of plant resistance, a method of caging them over the plants is necessary. A number of cypress flats 14×22×3 inches were constructed for growing the test plants. A space-planting arrangement for these flats was worked out and a pegboard constructed which, when pressed into the soil, provided seven rows of 12 holes, $1\frac{3}{4}$ inches apart. Seed of Spanish sweetclover was planted in the outer row of holes on all sides, and 50 holes remained for the test plants. Cages 15 inches high, of 28-mesh Lumite screen with hinged tops, were made to fit on the top edges of the flats (fig. 1). A 1-inch metal flange extending from the bottom edge of the cage into the soil along the inner sides of the flat prevented escape of the weevils. No weevils or weevil injury was ever observed on uncaged plants growing in the same greenhouse section with the caged plants. Recovery of weevils from the cages ranged from 90 to 95 %, indicating only normal mortality.

Preliminary studies were made to determine the most desirable stage of plant growth and the optimum duration of infestation. Infestations of weevils were made when the plants were in the unifoliolate, first-trifoliolate, and third-trifoliolate leaf stages. The amount of leaf loss per day varied with the growth stage and number of weevils used. Plants in the unifoliolate leaf stage infested with only one weevil per plant were completely destroyed in 1 day. The most nearly uniform plant growth was observed at the first-trifoliolate leaf stage. Older plants varied considerably in height and number of leaves present at the time of infestation. The weevils were removed when the first plant was completely destroyed. When infested in the first-trifoliolate leaf stage, this time interval was 3 to 4 days.

A reasonably rapid method of estimating percentage loss of leaflet area was developed by visually dividing the unifoliolate leaf and each leaflet of the trifoliolate leaves into four sections, utilizing the midrib of the leaflet as one dividing line. The percentage of area eaten was estimated separately for each section on all leaves having fully expanded leaflets and the average loss per plant was then calculated. The ability of an observer to make reliable ratings was determined by making three readings at intervals of 20 minutes on a flat of plants selected at random. The position of the flat on the bench was changed so that the observer did not make the readings in the same sequence each time. No significant differences were obtained between the three readings.

The number of weevils to be used per flat and the statistical soundness of the procedure were determined through

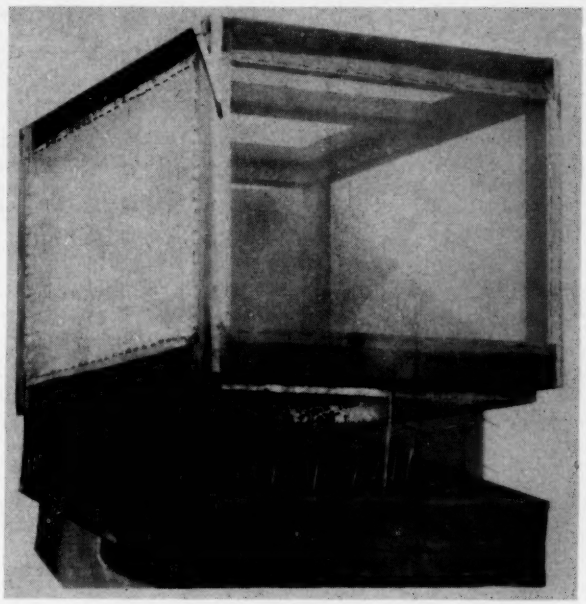

FIG. 1.—Cage used in confining sweetclover weevils over sweetclover plants. Cage raised to show flange around bottom.

replicated tests with 1, 2, 4, and 6 weevils per plant. The 50 test holes were planted to seed from a single weevil-susceptible plant (C-631) of *Melilotus alba* Desr. derived from four generations of self-pollination of individual plants. Since the calculated homozygosity of this selection is approximately 94% any differences in feeding damage may be assumed to be due primarily to chance or to the position of the plants in the flat, rather than to genetic differences. When all the test plants had one fully developed trifoliolate leaf, the border plants were removed, the flat was caged, and weevils that had been taken out of cold storage the previous day were introduced. When the first plant was completely defoliated, the weevils were removed and readings made within 1 day.

In a single flat one might test 10 plants of each of 5 varieties or 5 plants of each of 10 varieties. Replication could be achieved within the flat or by having more than one flat of a variety. By use of the analysis-of-variance technique the 50 plants in each flat were analyzed as 5 rows of 10 plants, 10 rows of 5 plants, and as two 5×5 Latin squares.

RESULTS.—The results of these analyses are presented in table 1. Significant differences between rows, and also between rows and columns in Latin squares, were found in most of the flats, regardless of the number of weevils used. There were also significant differences when the 2 outside rows were compared with either the 3 or 8 inside rows, feeding being heavier on the outside rows.

When the data obtained by the different experimental designs were compared, no significant differences were noted between varieties regardless of the number of weevils used. The standard errors of varietal means for each design are summarized in table 2. The 5 varieties in Latin squares gave the lowest standard errors regardless of the number of weevils used, and 10 varieties replicated 5 times gave the highest. One may conclude, therefore, that

Table 1.—Mean squares for rows and plants in a comparison of methods of randomizing sweetclover plants in caged flats infested with adult sweetclover weevils.

	5 Rows of 10 Plants			10 Rows of 5 Plants			Two 5×5 Latin Squares			
Flat No.	Between Rows	2 Outside vs. 3 Inside Rows	Among Plants Within Rows	Between Rows	2 Outside vs. 8 Inside Rows	Among Plants in Rows	Between Squares	Between Rows in Squares	Between Columns in Squares	Among Plants in Rows and Columns
				Six weevils per plant						
1	647	2460*	541	538	708	552	93	594	360	600
2	2781**	8923**	543	1715**	2835*	503	835**	886**	1461**	264
3	303**	1001**	75	175*	262	76	640**	117*	207**	43
				Four weevils per plant						
1	1699**	5462**	356	848*	1112	379	3322**	539	369	382
2	1850**	5452**	417	975*	2941*	434	599	1022**	1218**	238
3	1786*	2208*	478	925	6238**	508	229	1012*	965*	394
				Two weevils per plant						
1	634	2022**	246	486	1388*	231	1659**	340	402	188
2	1940**	5034**	263	366	224	408	800	312	1224**	204
3	1799**	5391**	456	827	2599*	507	155	911**	1610**	214
				One weevil per plant						
1	2463**	9429**	397	324	340	620	269	331	1515**	395
2	1536	2688	720	1440*	7863**	643	2023	1368*	1002	553
3	3014**	3542*	653	745	1972	868	959	718	2281**	515

* Significant differences at 5 per cent levels.
** Significant differences at 1 per cent levels.

the duplicate Latin-square arrangement gives greater precision than the other methods tested.

Table 2.—Percentage leaflet loss per flat and standard errors for varietal means obtained in a comparison of methods of randomizing sweetclover plants in caged flats infested with adult sweetclover weevils.

	Per Cent of Leaflet Loss		Standard Error		
Flat No.	Range	Average	10 Varieties with 5 Replicates	5 Varieties with 10 Replicates	5 Varieties in Two 5×5 Latin Squares
			Six weevils per plant		
1	29–100	72	10.4	7.4	7.7
2	9–100	60	10.4	7.1	5.1
3	64–100	90	3.9	2.8	2.1
Mean	34–100	74	8.2	5.7	4.9
			Four weevils per plant		
1	35–100	71	8.4	6.2	6.2
2	16–100	67	9.1	6.6	4.9
3	12–100	64	9.7	7.1	6.3
Mean	18–100	67	9.1	6.6	5.8
			Two weevils per plant		
1	32–100	73	7.0	4.8	4.3
2	24–100	71	7.3	6.4	4.5
3	15–100	65	9.5	7.1	4.6
Mean	24–100	69	7.9	6.1	4.5
			One weevil per plant		
1	17–100	64	8.9	7.8	6.3
2	9–100	54	12.0	8.0	7.4
3	3–100	58	11.4	9.3	7.1
Mean	10–100	59	10.8	8.4	6.9

The lowest standard errors were noted where two weevils per plant were used in the Latin square. The one low standard error observed for flat No. 3 where six weevils per plant were used is due in part to the fact that 90% of the leaflets were consumed before the weevils were removed. With this one exception, the errors are homogeneous and directly comparable.

Discussion.—From the tests described above it is obvious that any design with all plants of a variety in one row would confound the results. Although any of the randomized designs could be used for varietal testing, the Latin-square arrangement would be the most efficient.

For routine testing one of the varieties in each flat should be a check. At the present time there is available at the Nebraska Agricultural Experiment Station a weevil-susceptible line of plants that is approximately 96% homozygous. A single plant of this line, derived from line C-659, will be maintained by vegetative cuttings, and self-pollinated seed that will be approximately 98% homozygous will be used as a standard check in flats tested in the future. This procedure will provide a firm basis for comparisons from flat to flat and from year to year.

Other designs, such as a 5×5 lattice square or a 5×5 balanced lattice, might be used to test more varieties simultaneously. However, only two check plants would appear in each flat unless more than one of the 25 varieties were checks, in which case the variety means would not be estimated with the same precision as the checks. In the duplicate Latin square there would be 10 plants of the check and of each variety per flat, and, since all readings are related to the check, any differences between flats would be minimized and comparisons between varieties would be more accurate. Weevils should be caged

over the plants when all of them have one fully developed trifoliolate leaf, and should be removed when the first of the standard susceptible check plants is completely defoliated. Readings should then be made within 1 day.

It is realized that the use of only four varieties per flat in the duplicate Latin square severely limits the comparison between varieties, because of differences in selection pressure which may occur, depending on the lines or species present. To overcome this limitation, the best selections from different flats will be retested and compared in the same flat. At this time it appears desirable that large differences between entries should be sought. Germ plasm from varieties, species, or genera offering such differences can then be incorporated into a breeding program in an attempt to transfer these differences to adapted varieties. Any successful crosses can then be further evaluated.

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Evaluation of Representative California Spray Oils against Citrus Red Mite and California Red Scale[1]

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ABSTRACT

Laboratory spray trials were conducted to determine LD-95 values for several representative California light-medium-grade and medium-grade foliage spray oils against citrus red mite (*Panonychus citri* (McG.)) eggs and adult female California red scale (*Aonidiella aurantii* (Mask.)). Correlations of LD-95 values and physical-chemical properties of the oils are discussed.

The results obtained lead to the following conclusions. The magnitude of the LD-95 of the oils that were most efficient against citrus red mite eggs is about half that of the LD-95 of the same oils against adult female red scale. The killing action of light-medium-grade and medium-grade California spray oils against citrus red mite eggs is associated with the highest boiling portion of the oil. Five of the 8 oils tested were more efficient against citrus red mite eggs and 7 of the 8 oils were less efficient against adult female California red scale than was expected on the basis of respective values for average molecular weight.

The efficiency of two series of narrow-boiling petroleum fractions, one of highly naphthenic oil and the other of highly paraffinic oil, against eggs of the citrus red mite, *Panonychus citri* (McG.), and against adult female California red scale, *Aonidiella aurantii* (Mask.), was determined in laboratory experiments by Riehl & LaDue (1952). Comparable data for several representative California light-medium-grade and medium-grade foliage spray oils are reported here.

MATERIALS AND METHODS.—Samples of light-medium-grade and medium-grade California foliage spray oils were obtained from each of four oil companies engaged in the production of spray oils for the market in California. (For convenience, the four companies and the respective oils obtained from them are referred to by the letters A, B, C, and D.) The various oil samples represent highly refined petroleum fractions that comply with California spray oil specifications (Erb 1932, Marshall 1932). Values for average molecular weight and for 5% and 95% levels of fractional distillation are listed in table 1. Differences in various properties of spray oils within the same grade are permissible within a range listed for a particular specification. The oils of each grade from companies A, B, and D are highly naphthenic in character, *i.e.*, they have 50% chain-type reaction (Pearce & Chapman 1952); the two oils from company C have 59% chain-type reaction.

Descriptions of the general procedures used in the laboratory experiments may be found in publications by Riehl & LaDue (1952) and Riehl *et al.* (1953). The method of probit analysis used was changed to that given by Finney (1947), in which the Q/Z weighting coefficient

Table 1.—Molecular weight and fractional distillation properties of the experimental oils.

Oil	Average Molecular Weight	Distillation Temperature, °F. 5% Level	95% Level	Range Difference
Light-medium				
Company A	278	559	704	145
B	263	575	716	141
C	273	571	734	163
D	260	562	728	166
Medium				
Company A	292	579	707	128
B	290	589	722	133
C	295	573	746	173
D	287	572	752	180

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[2] The authors express their appreciation to the California Spray-Chemical Corporation for determinations of the physical and chemical properties of the California spray oils used in these experiments.

for adjustment of natural mortality was included as a factor in the maximum likelihood estimate calculations. The precision of the experiments was thereby sufficiently improved that 12 to 14 dosage-mortality points for each oil provided confidence limits as good *as* or better than those obtained in earlier work with 21 to 30 points. For general measures of efficiency it appeared that the various oils could be tested independently of each other in the chronological course of the laboratory experiments. Therefore, applications of the sprays for the dosage levels and replications for any given oil were all made in a single day.

DISCUSSION OF RESULTS.—Values for the LD-95 and for the regression lines of the probit analyses of the results of the laboratory spray trials are presented in table 2. The coefficient of X in the regression equations is the value of *b*, the regression coefficient or the slope of the line. The oils are arranged in table 2 within each of the grades in increasing order of LD-95, or decreasing order of efficiency against citrus red mite eggs.

Extensive determinations were made of the physical-chemical properties of the spray oils used in the experiments; the determinations of properties made available included 19 separate properties for each oil. Careful study of correlations of LD-95 values of table 2 with differences within the range of values of given properties failed to bring out consistent trends, although differences in the LD-95 values against citrus red mite eggs are relatively large. Since differences between the oils within various properties are relatively small and no important contribution to the interpretation of the results could be found in most of the properties, no complete listing of the determinations of the various properties is made in this paper.

If the LD-95 values of table 2 for citrus red mite eggs are entered on the curve of efficiency vs. molecular weight of the naphthenic narrow-boiling petroleum fractions for citrus red mite eggs given by Riehl & LaDue (1952), it will be seen that the intersection with the curve occurs below the critical molecular weight value and on the portion of the curve in which efficiency is decreasing rapidly with decreasing molecular weight. In this portion of the curve, small differences in molecular size are associated with large differences in efficiency. Another aspect of this portion of the curve is the likelihood that variability of the dosage-mortality response will increase as the efficiency decreases. Poor confidence limits and reproducibility of the LD-95 values for citrus red mite eggs listed in table 2 would not be unexpected at the efficiency level found.

Eleven of the properties determined for the experimental oils are measures of molecular size. Comparisons of oil D with oils A or B of either grade showed that differences in 7 of these 11 properties indicated smaller molecular size for oil D.

Against citrus red mite eggs, the California spray oils used here were more efficient than was expected from correlation with molecular weight for narrow-boiling naphthenic fractions (Riehl & LaDue 1952); properties of the narrow-boiling fractions are given by Pearce & Chapman (1952). The distillation ranges of the California spray oils used here are wider than those of the narrow-boiling naphthenic fractions by factors of 4 to 6 times. As indicated by the values listed in table 1 for 95% distillation, the complete data for fractional distillation show that the upper 10 to 15% of the oils distill at temperatures comparable to those of the narrow-boiling naphthenic fractions 8 and 9 with molecular weights of 323 and 337, respectively. This evidence indicates that the killing action of light-medium-grade and medium-grade California spray oils against citrus red mite eggs is associated with the highest boiling portion of the oil.

The order of arrangement of the LD-95 values against citrus red mite eggs in table 2 seems to be associated as well with the magnitude of the difference between the 5% and 95% distillation values of table 1 as with any other property of the oils. The order of arrangement is the same for the medium-grade oil. The only exception is light-medium oil C. This may be influenced by two factors: (1) oil C is higher in paraffinicity than the other oils, and (2) light-medium oil C is narrower in distillation range than medium oil C. Part of the increase in the LD-95 of the less efficient oils for citrus red mite eggs may be due to a larger ratio of inefficient to efficient portions of the oils with wider distillation ranges; *i.e.*, the amount of the efficient portion of the oil required for 95% kill is accompanied by a larger volume of inefficient oil for the oils of wider distillation range. Thereby, in micrograms of total oil the LD-95 is greater for the oils of wider distillation range. The validity of this hypothesis is subject to the

Table 2.—Efficiency of several light-medium-grade and medium-grade California foliage spray oils against day-old eggs of citrus red mite and against adult female California red scale.

	Day-Old Eggs of Citrus Red Mite			Adult Female California Red Scale		
Oil	LD-95, in Micrograms Oil/Sq. Cm.	Confidence Limits, in % LD-95	Probit Analysis[a] $Y=a+b(X-\bar{x})$	LD-95 in Micrograms Oil/Sq. Cm.	Confidence Limits, in % LD-95	Probit Analysis[a] $Y=a+b(X-\bar{x})$
Light-medium						
C	48.7	12.3	$=3.78X+0.26365$	107.5	6.9	$=3.88X-1.24454$
B	68.2	8.3	$=3.23X+0.72808$	109.0	9.8	$=3.39X-0.27250$
A	93.9	8.9	$=3.25X+0.22916$	101.5	6.4	$=4.73X-2.84511$
D	118.3	25.6	$=2.05X+2.39984$	108.2	5.4	$=4.15X-1.80038$
Medium						
A	47.2	23.6	$=2.48X+2.48564$	89.7	5.7	$=3.73X-0.63451$
B	50.7	9.9	$=2.48X+2.41599$	105.9	6.3	$=4.52X-2.50304$
C	67.8	13.4	$=2.74X+1.62062$	115.4	10.5	$=3.71X-1.00585$
D	94.2	24.8	$=1.86X+2.96991$	113.4	6.5	$=3.91X-1.38981$

[a] Y = probit value for per cent mortality.
X = $\log_{10}$ of oil deposit in micrograms oil per sq. cm. of fruit surface.

criticism that temperatures for 95% distillation are higher for the wider-range oils and that the increase in range is greater at the upper end than at the lower end of the wider-range oils.

In table 2 the LD-95 values of the light-medium-grade oils for red scale are as good as would be expected from comparison of their average molecular weights with the correlation of efficiency vs. molecular weight for naphthenic narrow-boiling fractions against red scale (Riehl & LaDue 1952). The medium-grade oils are not as efficient as was expected from their molecular weights. The curve for the narrow-boiling fractions indicates that the maximum efficiency of oil of naphthenic composition against adult female California red scale occurs at an average molecular weight of 290. Data from Pearce & Chapman (1952) show that the molecular size is found in a narrow boiling range at temperatures near 650° F., at 760 mm. pressure. Seventy per cent of the light-medium-grade oils and of medium oil A, 60% of medium oils B and C, and 50% of medium oil D of table 1 were distilled at 650° F. The higher efficiency of medium oil A in the laboratory trials may be associated with its narrower distillation range and with its having less material at either the lower or the upper end of the boiling range, or at both ends, than any of the other oils tested. Against adult female red scale inefficient portions of the oil occur at both ends of the distillation range. Medium oil A may have less inefficient oil per unit of efficient oil, and less total oil may therefore be required for 95% kill. While the evidence is satisfactory for the above interpretation of the laboratory results, portions of spray oils boiling above 650° to 660° F. are important to the residual effect of oil spray in the overall control of red scale in the field (Ebeling 1950, Riehl & Carman 1953).

Consistent trends are not evident in correlations of the LD-95 values of table 2 for red scale and in differences in the physical-chemical properties determined for the various oils. It may be reasonable to assume that the differences in the results of table 2 for red scale are not critical and are within the limits of observed differences in the properties of the oils and the variation inherent in the experimental procedure.

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Chemical Control of Clover Leaf Weevil Larvae[1]

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ABSTRACT

Larvae of the clover leaf weevil (*Hypera punctata* (F.)) were a serious pest of legumes in Southwest Kentucky during the spring of 1956. The usually prevalent fungus disease of this insect did not give satisfactory control. Field tests in combination with laboratory studies indicated that the larvae were relatively difficult to kill within a short time with economically feasible dosages of various insecticides. However, aldrin, Chlorthion, endrin, heptachlor, lindane, and toxaphene, all applied as emulsions, caused a high per cent of moribund individuals. These moribund larvae did little feeding and most of them were not able to complete development. Such larvae were commonly found in open spaces away from the host plant, and their numbers were reduced, presumably by predators. Diazinon and methoxychlor emulsions apparently had little effect on the larvae. The treatment with malathion emulsion gave the highest mortality of larvae soon after application but more larvae survived in this treatment than in some of the other treatments when the insects were held in laboratory cultures for several weeks. Granulated formulations of aldrin and heptachlor were far inferior to emulsions of the same materials used at comparable levels.

Larvae of the clover leaf weevil, *Hypera punctata* (F.), are ordinarily present each spring in legume fields of North Central Kentucky. Seldom is applied control necessary as there is usually an epizootic fungus disease attacking the larvae in this region. During the relatively cool, dry months of March and April in 1956, the clover leaf weevil was again of little economic importance in North Central Kentucky but it was a suprisingly serious pest of legumes in the southwestern part of the state, a region where the insect was previously almost unknown to farmers. There was an estimated average of as high as 40 larvae per square foot in some red clover fields. Some stands were so badly damaged that it seemed expedient

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director. Accepted for publication October 18 1957

to plow them under. Alfalfa was severely damaged in some fields while Ladino clover and lespedeza were attacked to a small extent. Damage first became apparent about the middle of March. Entomophthorous fungi did not give indications of controlling the insect as less than 1% of the larvae were obviously parasitized in the fields examined. Insecticide experiments were instituted as an effective control was badly needed and there seemed to be little published data on spring applications of chemicals to control the pest.

In March of 1957, clover leaf weevil larvae were again numerous and damaging in the southwestern part of Kentucky, and again field tests with insecticides were conducted. However, during the first part of April the infested area had frequent rains and prevailing high humidity which presumably encouraged the fungus disease of the larvae to such an extent that mortality was as high as 80% in untreated check plots 1 week after the treatments were applied.

MATERIALS AND METHODS.—Field experiments in 1956 consisted of a small-plot, hand-treated test and a large-plot, tractor-treated test. All insecticides were commercial formulations prepared by the basic manufacturer. All field plots were located in Simpson County, Kentucky.

Small-plot experiment.—This test in a red clover field consisted of 13 treatments in a randomized complete block design of three replicates. The plot size was 10×10 feet. There were nine treatments with emulsions and three with granulated formulations, all of which were applied April 17, 1956. The emulsion concentrates were diluted with water and applied at a rate of 20 gallons of spray per acre with a 1-gallon "B & G" knapsack sprayer having a Tee-Jet number 730039 nozzle. Granulated insecticides were mixed with quartz sand to increase the bulk, and spread by hand as evenly as possible over the ground surface. The dosages of the chlorinated hydrocarbon insecticides were kept low so as to be economically feasible on a field basis. The organophosphates were used at levels effective for control of the pea aphid (*Macrosiphum pisi* (Harris)), a pest sometimes occurring about the same time as the clover leaf weevil. Insecticides and dosages used are shown in table 1.

The average maximum temperature during the test was about 61° F. and there was approximately ½ inch of rainfall. There was little wind while the insecticides were being applied.

To evaluate the treatments, the per cent mortality, the feeding activity of larvae taken from the plots, and the number of larvae per plant were recorded. The per cent mortality was obtained by counting the first 50 clover leaf weevil larvae seen in each plot. Counts were made 2 days and 1 week after the plots were treated. As it was difficult to decide quickly if a specimen was poisoned, the larvae from each plot were placed in a ventilated ice-cream carton and taken to the laboratory for more critical observation. The insects transported and stored in these containers were regularly given untreated red clover as food. The average percentage of dead larvae in the treatments 2 days after application, and the average percentage of dead, moribund, and healthy larvae 1 week after treatment are given in table 1.

Fifty larvae collected at random from each plot 1 week after treatment were used in laboratory tests to determine the effects of the various treatments on the feeding activity of the specimens. The larvae were starved for 12 hours and then given leaves of supposedly insecticide-free red clover. A ratio of one entire leaflet to five larvae was

Table 1.—Results from a small-plot test with insecticides for the control of clover leaf weevil larvae in Simpson County, Kentucky, 1956.

Insecticide and Formulation[a]		Dosage[b]	Two Days After Treating[c]	One Week After Treating[c]				
			Per Cent Dead Larvae	Per Cent Larvae			Per Cent Leaf Surface Eaten	Number Larvae Around Plants[d]
				Dead	Moribund	Healthy		
Heptachlor	E	0.25	46	20	68	12	16	2.5
Heptachlor	G	0.25	24	17	30	53	61	9.0
Aldrin	E	0.25	37	16	58	26	25	3.1
Aldrin	G	0.25	18	5	6	89	92	14.1
Dieldrin	G	0.125	16	4	4	92	98	13.0
Endrin	E	0.125	33	11	50	39	76	5.7
Lindane	E	0.125	33	13	13	74	63	3.1
Toxaphene	E	1.0	34	23	24	53	37	2.7
Methoxychlor	E	0.5	8	3	6	91	98	10.7
Malathion	E	0.75	50	46	16	38	29	2.9
Chlorthion	E	0.25	43	18	15	67	74	1.3
Diazinon	E	0.25	10	1	3	96	95	13.9
Untreated	—	—	6	1	2	97	99	14.3
L.S.D. at 5% level			14.1	9.6	13.0		35.5	4.0
at 1% level			19.8	13.5	18.2		49.7	5.4

[a] E, emulsion; G, granules.
[b] Pounds of active ingredient per acre.
[c] Each figure represents the mean of three replicates.
[d] Larvae were counted around five red clover plants in each replicate.

used. After 24 hours the percentage of eaten leaf surface was estimated as given in table 1.

The number of larvae around five clumps of red clover in each plot was recorded 1 week after applying the treatments. Only the live individuals on the plants or on the ground within a 6-inch radius of the base of the plants were included in the counts. These results are also given in table 1.

Fifty additional living larvae from each plot were retained in a carton at room temperature with food and observed at weekly intervals for 5 weeks after treatment to determine the development of the insects. The average percentage of adults, pupae and larvae, and the percentage mortality for each treatment are given in table 2.

Large-plot experiment.—Nine treatments with emulsions were applied to Atlantic alfalfa on April 20 and 21 with a tractor-drawn sprayer having a cluster nozzle which delivered 12 gallons of water per acre at 50 p.s.i. Each plot was about 5 acres in size with no replication. Pre-spray counts of the clover leaf weevil indicated that the infestation was fairly evenly distributed throughout the field and that there was an average of about 18 larvae per square foot.

Treatments were evaluated on April 23 by taking 100 larvae at random from each plot. These were put into a labelled carton, given food, and taken to the laboratory where they were categorized the following day into healthy, moribund and dead individuals. The moribund and healthy larvae were then put on potted red clover in cages and examined after 2 weeks and again after 1 month. The results of this test are given in table 3.

The highest temperature reported between time of application and evaluation of the treatment was 71° F. No rainfall was recorded.

RESULTS AND DISCUSSION.—The larvae of the clover leaf weevil seemed to migrate very little either within or between plots. Also, the infestation was fairly uniform over a field if the stand of the legume was reasonably uniform. During the period of the tests, all larval stages were represented in the fields. No appreciable difference was noticed among the various instars in susceptibility to poisoning by any of the insecticide treatments. Prepupation was first noticed on April 19, and by May 4 about 3% of the field population had formed cocoons. Adults were appearing by May 4 but they never built up to large populations even in fields heavily infested with the larvae. On June 28, there was less than one adult per sweep with an insect net in first-cutting alfalfa that had had an average of 26 larvae per square foot.

Table 2.—Development of clover leaf weevil larvae taken from hand-treated insecticide plots 1 week after application and kept for 1 month in laboratory cultures, 1956.

INSECTICIDE[a]	PER CENT[b]			
	ADULTS	PUPAE	LARVAE	DEAD
Heptachlor	2	5	3	90
Heptachlor	11	11	2	76
Aldrin	5	4	0	91
Aldrin	45	15	2	38
Dieldrin	22	14	2	62
Endrin	19	7	3	71
Lindane	14	15	1	70
Toxaphene	15	9	6	70
Methoxychlor	50	12	0	38
Malathion	26	3	1	70
Chlorthion	12	5	1	82
Diazinon	43	11	5	41
Control	37	15	10	38

[a] Dosages and formulations of insecticides are the same as those listed in table 1.
[b] Each figure represents the mean of three replicates of 50 larvae each.

Small-plot experiment.—The mortality counts from the hand-treated plots both 2 days and 1 week after treatment showed that several of the insecticide treatments gave larval kills significantly higher than the average mortality in the control. The exceptions were with the treatments with aldrin and dieldrin granules, and with methoxychlor and Diazinon emulsions which did not result in kills significantly higher than in the untreated control at the 5% level. However, as shown in table 1, the highest average mortality for any treatment in these tests was only 50%. There were fewer dead larvae in all treatments in the 1-week collection than in the 2-day collection.

Table 3.—Results from a large-plot field test with insecticides for the control of clover leaf weevil larvae in Simpson County, Kentucky, 1956.

TREATMENT[a]	DOSAGE[b]	TWO DAYS[c]			TWO WEEKS[c]				ONE MONTH[c]			
		Per Cent Larvae			Per Cent Alive			Per Cent Dead	Per Cent Alive			Per Cent Dead
		Healthy	Moribund	Dead	Adults	Pupae	Larvae		Adults	Pupae	Larvae	
Malathion	12	18	43	39	1	6	9	84	8	0	3	89
Heptachlor	4	4	91	5	0	6	7	87	0	1	2	97
Heptachlor	6	2	94	4	1	5	6	88	2	0	0	98
Endrin	2	6	92	2	0	5	6	89	1	3	3	93
Endrin	3	5	91	4	0	6	12	82	1	5	3	91
Toxaphene	16	8	88	4	0	11	13	76	2	7	5	86
Toxaphene	24	9	85	6	0	9	10	81	0	4	2	94
Lindane	2	56	44	0	0	13	23	64	2	4	4	90
Lindane	3	17	82	1	0	6	12	82	4	2	5	89
Control	—	97	2	1	2	46	29	23	24	25	9	42

[a] All insecticides were commercial formulations of emulsion concentrates.
[b] Dosages are given in ounces of active ingredient per acre.
[c] Each figure represents the mean of five subsamples from an unreplicated plot.

Although the actual kill of larvae was small, some treatments in comparison with the check had many larvae that were more wrinkled and deeper green in color. Such abnormal-appearing larvae were commonly found in open spaces exposed to direct sunlight. These were recorded as moribund.

In the treatments with emulsions of heptachlor, aldrin, and endrin 50% or more moribund larvae were recorded 1 week after their application. In general, the treatments with the highest kills had the greatest number of moribund individuals. However, malathion ranked first among the treatments in percentage kill but ranked sixth in percentage moribund.

The laboratory feeding test with larvae collected 1 week after treatment showed that moribund individuals did little feeding during the first 24-hour period. There was a direct correlation between the percentage of healthy larvae and the percentage of leaf surface eaten (table 1). The adverse influence on larval feeding produced by some of the insecticides applied in these tests would seem to indicate that practical control from their use is possible although the kill obtained with these same materials at the dosages used was not appreciable.

There were significantly fewer larvae surrounding plants in some treatments than in others 1 week after application. The treatments with fewer larvae were, in general, the ones that were considered as the more effective by other criteria. Perhaps predators of the weevil were better able to attack the moribund larvae which were accessible in unprotected areas.

The retention of the larvae from the hand-treated plots in laboratory cultures indicated that most of the individuals in some treatments failed to complete metamorphosis and eventually died. Emulsions of aldrin and heptachlor gave the lowest survival rate. Malathion gave the highest kills 2 days and 1 week after applying the treatments but 5 weeks after application there were more healthy individuals in this treatment than in some of the other treatments. At the dosages used some insecticides apparently were not effective toxicants against the clover leaf weevil.

In comparison with emulsions, the treatments with granulated aldrin and heptachlor at comparable dosages were inferior in control of the larvae. The emulsions were among the most promising treatments, whereas the granulated-insecticide treatments gave results which were not significantly different from those of the untreated plots. The reason for this wide difference in effectiveness of the two types of formulation is not known.

Large-plot experiment.—The large plots furnished additional evidence that the larvae of the clover leaf weevil were comparatively difficult to kill with the insecticides tested as most of the treated areas had few dead individuals. As in the hand-treated plots there were, however, many larvae that were considered moribund. The 10-ounce-dosage of lindane did not appear to give as good control as the other treatments (table 3). Lindane was the only material that indicated a difference in effectiveness between the high and low dosages. Malathion again gave the highest average mortality 2 days after spraying; but the malathion-treated plot also had a higher average number of healthy larvae than some of the other treated plots.

When taken to the laboratory, most of the individuals taken from the field plots treated with insecticides died in the larval or pupal stage. Thus, there were no appreciable differences among the various insecticide treatments in their influence on metamorphosis. However, significantly more larvae from the check plots completed development than from the insecticide-treated plots. This would indicate that, although at the dosages used the insecticides gave poor kills 2 days after application, they had a detrimental effect on metamorphosis.

Biological and Chemical Studies on the Decline of Soil Insecticides[1]

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ABSTRACT

Special studies were made with two different soils when aldrin, dieldrin and heptachlor (5 p.p.m.) were mixed with regular and autoclaved soils with and without the addition of urea at 5 p.p.m.

The rate of decline of aldrin, dieldrin and heptachlor was measured by total chlorine and bioassay methods. In addition, decline of aldrin and dieldrin was measured by the specific phenylazide method.

In general, the total chloride and bioassay methods showed a high initial recovery of the insecticides from the soil, followed by a rapid decline. The specific phenylazide method showed little insecticide change after 11 months.

The decline of insecticides could not be associated with soil microörganisms or with the physical and chemical properties of the soil.

Chlorinated hydrocarbon insecticides such as DDT, aldrin, dieldrin, heptachlor and others, have been used commercially as soil insecticides for control of many troublesome soil pests. In Oregon, Morrison & Crowell (1952) showed that a number of materials gave almost

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perfect control of the tuber flea beetle, *Epitrix tuberis* Gent., and certain species of wireworms, *Limonius infuscatus*, Mots., *L. californicus* Mann., and *L. canus* Lec., for 3 years. They later reported (1957), that when aldrin or dieldrin was applied at the rate of 10 pounds of toxicant per 6-inch acre, control was maintained for an 8-year period. Heptachlor was following a similar pattern but had been tested for only 6 years. At lighter dosages (2 to 5 pounds per 6-inch acre) these materials were giving excellent control for 4 years.

During the period 1949–52, these materials were also evaluated for the control of the cabbage maggot, *Hylemya brassicae* (Bouché) on turnips. Aldrin and heptachlor, at the 10-pound rate, gave good initial control but declined in effectiveness after 2 years. Dieldrin, not impressive during the first and second year, improved in effectiveness in the third and fourth season and then declined.

Soils from these treated plots were analyzed by Terriere & Ingalsbe (1953) in 1950 and 1951. In this work, there appeared to be little agreement between the total chlorine method of chemical analysis and the bioassay evaluations. There was no suggestion of chemical transformations into more toxic components.

During the period 1950–54, numerous field trails were made with aldrin, dieldrin and heptachlor to determine their effectiveness for control of the garden symphylid, *Scutigerella immaculata* (Newp.) In some instances, good commercial control was obtained while others resulted in failures. In order to obtain information on factors responsible for these anomalous results, special studies were started in 1954.

PROCEDURE.—A number of concrete cells, open at top and bottom, were constructed at the Entomology Farm, Corvallis, Oregon. They measured 3 ft. by 1½ ft. by 2 ft. deep. Below the 2-foot depth, 1 foot of gravel was placed to provide good drainage.

Two different soils were used. One, classified as Chehalis silt loam, was from an area where successful symphylid control had been accomplished. The other, classified as Olympic silt loam, was from an area where aldrin, dieldrin and heptachlor had not contributed to symphylid abatement.

The soil from each area was obtained from ½- to ¾-acre plots by taking random samples of soil and sifting them through a ¼-inch mesh screen on a mechanical sifter. Three cubic yards of soil from each location were brought to the farm and placed in a holding box. In order to obtain a uniform soil, the contents of each box were mixed separately in a large (10-cubic-yard) concrete mixer for 15 minutes, and then returned to the boxes. There was sufficient water in the mixer to cause the dry soil to form small pellets. These were removed by again sifting the soil through the ¼-inch mesh screen.

Two hundred fifty pounds of air-dried Chehalis soil were needed to fill each cell (6.27 cu. ft.) to the desired level. Three hundred pounds of Olympic soil were needed to give an equal volume.

In one series of treatments aldrin, dieldrin and heptachlor were mixed with each of these soils at the rate of five parts toxicant per million by weight,[6] in a medium size (¾-cu.-yd.) concrete mixer. In another series, urea at 5 ppm. was mixed with these insecticides and soils in order to determine if it had a stabilizing influence on the insecticides. In a third series of mixtures, the insecticides were added to the soil after it had been autoclaved in order to eliminate, at least for a while, the action of soil microörganisms on the toxicants. Check plots, with and without urea, were provided. Dusts containing 1% of the respective desired materials were used in making these soil treatments in order to provide thorough distribution.

The treatments were unreplicated because it was physically impossible to handle over three tons of soil on four different occasions and still provide for replication of treatments. Since the trials were exploratory in nature and since uniform soil samples and ideal insecticide mixing were provided, the necessity of having replications was minimized.

The cells were not planted to crops and were kept relatively free from weeds. The few weeds which grew were pulled and returned to their respective cells. Occasionally the soil crust was broken but no thorough stirring of the soil was done.

Physical, chemical and microbial analyses were made on these soils prior to mixing them with insecticides.

The first samples of soil from the different treatments were taken immediately after mixing. Subsequent samples were taken from the top 6 inches of soil after 8 and 11 months. They were placed in clean cardboard containers, using precautions against gross chemical and microbial contamination. These samples were used by the Department of Agricultural Chemistry for chemical analyses by the total chloride method and for bioassay tests with mosquito larvae for aldrin, dieldrin and heptachlor. In the 8-month sample, the 6-inch core was divided into two 3-inch cores for analyses of the top and bottom of the sample. This was done to determine if there was any difference in the rate of insecticide decline due to leaching.

Additional samples from 0 time, 1 month, 8 months, and 11 months were submitted to the Department of Bacteriology for microbial analyses and for chemical analyses by the phenylazide method.

Samples of the two soils were also sent to the Velsicol Corporation for special study on the stability of heptachlor. In these trials, heptachlor, at 200 and 300 parts per million, was blended with the soils and subjected to oven tests at 50° C. for a 2-month period. Using the colorimetric method of Polen & Silverman (1952), chemical analyses were made at intervals of 0, 30 and 60 days.

METHODS.—*Specific Analyses of Aldrin and Dieldrin.*—The phenylazide method of O'Donnell *et al.* (1954, 1955), was employed. The extraction procedure for aldrin and dieldrin was based on methods used by O'Donnell & Neal (1952). Soil samples were air-dried sufficiently to permit screening through a 10-mesh sieve and were stored in tightly closed screwcap jars. The moisture content in the samples ranged from 1.1 to 7.1%. For extraction, 20 grams of soil on a water free basis were placed in a pyrex bottle and a sufficient volume of redistilled Skellysolve B was added to give a solvent-soil ratio of 2:1 and 0.5:1. An aluminum foil stopper was inserted and the mixture was shaken 30 minutes on a rotary shaker. After allowing

[6] Subsequent analyses of the commercial aldrin dust revealed that the rate of application gave 5.4 parts per million of actual aldrin.

solid material to settle for 5 minutes, the resultant supernatent was clear. The volume of extraction solvent used and the size of the solvent aliquots taken were adjusted so that the calculated amounts of insecticide would fall approximately in the center of the standard curves having a range of from 0 to 40 micrograms.

The solvent used for dieldrin was 35 ml. Skellysolve B plus 5 ml. acetone for 20-gram portions of soil. Otherwise the procedure was the same as given for aldrin. The aldrin and dieldrin used for standards were recrystallized compounds of 99.5% or better purity, obtained from the Shell Chemical Corporation, Denver, Colorado.

Cleanup chromatography required for the analyses of fruits and vegetables to remove interfering pigments was unnecessary with soil extracts. Duplicate soil blanks were run in all cases so that an apparent aldrin or dieldrin value could be subtracted from the values of the treated soils.

Extraction for Bioassay and Total Chlorine Method.— One hundred fifty gram samples were extracted with 150 ml. of a 2:1 mixture of benzene and isopropanol by shaking in a centrifuge bottle for 15 minutes. After centrifuging, the clear supernatant liquid was decanted and stored in screwcap bottles until analyzed.

The Total Chlorine Method.— The method of Agazzi *et al.* (1953) was used for chlorine determinations. Aliquots containing approximately 200 micrograms of insecticide were concentrated to a small volume and combusted in a modified Braun sulfur apparatus. The resulting inorganic chloride was determined by amperometric titration, using 0.001 N silver nitrate solution as the titrant. Each day's work included mineral oil blanks, untreated soil samples, treated soil samples, and fortified samples. This was done to check the reliability of the method. Sensitivity of the method was determined to be 0.1 part per million.

Bioassay.—Mosquito larvae, *Culex quinquefasciatus* Say, were used in bioassay evaluations. Aliquots of soil extract containing approximately 3 micrograms of insecticide were evaporated to dryness under an air-jet and the residues redissolved in small amounts of acetone. Water was then added to obtain a proper dilution determined by a preliminary assay. The insecticide was suspended in this solution by vigorous shaking. Progressive amounts of the suspension were added to small vials together with 10 five-day-old larvae and the volume in each vial was made up to 15 ml. Each sample was assayed at four duplicate levels. Mortalities were determined after 24 hours. Each set of bioassays included untreated, treated, and fortified soil extracts. The concentration of the toxicant in treated soil extracts was determined by comparing dosage-mortality curves of the standards and unknowns. Sensitivity of the method was computed as 0.1 p.p.m.

Microbial Analyses.—Samples as collected were too moist to pass through a screen. After partial air-drying, moisture determinations were made and equivalents of 20 grams of soil on a water-free basis were used to prepare 1:5 dilutions in sterile water blanks. The initial dilution was shaken vigorously on a mechanical shaker for 10 minutes, after which coarse particles were allowed to settle for 5 minutes prior to making dilutions. Subsequent dilutions of 1:500 and 1:5,000 were plated with peptone-glucose acid agar for molds; 1:50,000 and 1:5,000,000 dilutions were plated with sodium albuminate agar for bacteria and streptomyces (Fred & Waksman 1928).

Counts were made as soon as colonies could be differentiated after incubation at 28° C. All dilutions were plated in triplicate and the arithmetic mean in each case was taken as an average.

Measurement of Physical Properties.—Mechanical analyses, soil moisture tension curves and moisture equivalent of the soil samples were determined by the Soils Department of Oregon State College. The U. S. Department of Agriculture pipette method of Kilmer & Alexander (1949), the pressure membrane and porous plate apparatus of Richards (1947, 1948) and the centrifuge method of Briggs & McLane (1907), respectively, were used in measuring these values.

RESULTS AND DISCUSSION.—There was considerable

Table 1.—Summary of microbial analyses from insecticide-treated soils.

Treatment	Interval After Treatment	Differential Mold Counts (Thousands)						Total Bacteria (Millions)
		Mucors	*Penicilla*	*Aspergilli*	*Trichoderma*	Others	Total	
Checks[a]	0 days	0.8	46.6	0.2	—	3.9	51.5	6.1
	1 month	6.0	116.6	0.9	2.9	9.8	136.2	9.8
	8 months	2.9	70.4	0.5	0.1	4.0	77.9	7.4
	11 months	0.6	48.2	0.1	1.9	11.7	62.5	7.1
Insecticides[b] and soil types	0 days	1.3	27.3	0.1	—	4.1	32.8	6.0
	1 month	5.0	176.2	—	6.4	11.0	198.6	14.6
	8 months	1.5	29.2	0.2	0.2	1.6	32.7	9.4
	11 months	2.2	43.8	0.5	1.8	8.8	57.1	6.8
Insecticides,[b] soil types and urea	0 days	1.3	20.0	0.2	—	4.9	26.4	4.1
	1 month	3.1	180.6	1.2	1.2	12.3	198.4	8.4
	8 months	1.5	38.8	0.2	0.4	0.4	41.3	5.0
	11 months	1.1	84.0	0.6	1.3	0.9	87.9	8.2
Insecticides[b] in autoclaved soil types	0 days	458.5	2792.1	—	—	199.4	3,450.0	136.0
	1 month	610.6	3523.3	8.0	—	115.6	4,257.5	120.6
	8 months	17.5	80.5	—	0.4	3.1	101.5	11.5
	11 months	20.0	233.9	—	7.0	2.9	263.8	12.0

[a] Mean of Olympic and Chehalis soils.
[b] Mean of aldrin and dieldrin in Chehalis and Olympic soils.

Table 2.—Percentage of aldrin recovered from four samplings of treated soil by three different analytical methods.

Treatment and Type of Silt Loam Soil	Interval Between Treatment and Sampling									
	0 Days			One Month	Eight Months			Eleven Months		
	Total Chlorine	Bioassay	Phenyl-azide	Phenyl-azide	Total Chlorine[a]	Bioassay[a]	Phenyl-azide	Total Chlorine	Bioassay	Phenyl-azide
Chehalis					84.3 72.6	65.7 52.8				
	99.1	99.1	22.2	13.0	78.5	59.3	27.7	32.8	31.5	16.7
Autoclaved Chehalis					73.7 85.9	67.6 77.8				
	95.9	96.3	35.1	33.3	79.8	72.7	42.6	85.2	36.1	22.2
Chehalis plus urea					80.7 76.7	63.9 42.6				
	85.7	87.0	22.2	14.8	78.7	53.3	22.2	24.1	18.5	20.4
Olympic					73.7 76.1	70.4 76.9				
	91.3	94.4	37.0	16.7	75.0	73.7	25.9	87.4	38.0	25.9
Autoclaved Olympic					77.2 83.7	66.7 75.0				
	111.1	108.3	27.8	29.6	80.5	70.9	44.4	43.5	44.4	38.8
Olympic plus urea					73.5 74.4	63.9 61.1				
	91.3	92.6	37.0	16.7	74.0	62.5	25.9	54.1	34.2	24.1

[a] The three percentages given in this column for each treatment and type of silt loam soil are for the top 3 inches, bottom 3 inches, and mean, respectively.

Table 3.—Percentage of dieldrin recovered from four samplings of treated soil by three different analytical methods.

Treatment and Type of Silt Loam Soil	Interval Between Treatment and Sampling									
	0 Days			One Month	Eight Months			Eleven Months		
	Total Chlorine	Bioassay	Phenyl-azide	Phenyl-azide	Total Chlorine[a]	Bioassay[a]	Phenyl-azide	Total Chlorine	Bioassay	Phenyl-azide
Chehalis					87.4 92.6	81.0 86.0				
	104.0	102.0	68.0	88.0	90.0	83.5	68.0	21.8	25.0	76.0
Autoclaved Chehalis					85.0 83.0	82.0 66.0				
	91.0	89.0	50.0	100.0	84.0	74.0	96.0	38.0	40.0	88.0
Chehalis plus urea					86.2 87.2	79.0 83.0				
	91.0	87.0	102.0	78.0	86.8	81.0	64.0	39.6	28.0	84.0
Olympic					88.8 89.2	71.0 81.0				
	99.8	95.0	58.0	90.0	89.0	76.0	72.0	31.2	32.0	82.0
Autoclaved Olympic					84.6 86.6	74.0 81.0				
	89.0	87.0	50.0	54.0	85.6	77.5	110.0	95.6	28.0	98.0
Olympic plus urea					95.6 90.0	80.0 79.0				
	92.4	99.0	68.0	58.0	92.8	79.5	62.0	31.0	38.0	72.0

[a] The three percentages given in this column for each treatment and type of silt loam soil are for the top 3 inches, bottom 3 inches, and mean, respectively.

Table 4.—Percentage of heptachlor recovered from three samplings of treated soil by two different analytical methods.

Treatment and Type of Silt Loam Soil	Interval Between Treatment and Sampling					
	0 Days		Eight Months		Eleven Months	
	Total Chlorine	Bioassay	Total Chlorine[a]	Bioassay[a]	Total Chlorine	Bioassay
Chehalis			92.4 88.6	74.0 64.0		
	107.4	104.0	90.5	69.0	93.4	31.0
Autoclaved Chehalis			91.0 93.0	73.0 73.0		
	97.0	101.0	92.0	73.0	112.6	36.0
Chehalis plus urea			91.2 93.0	84.0 79.0		
	108.4	107.0	92.1	81.5	91.6	35.0
Olympic			90.2 93.0	86.0 82.0		
	97.6	99.0	91.6	84.0	45.6	35.0
Autoclaved Olympic			100.0 105.0	88.0 89.0		
	104.4	105.0	102.8	88.5	33.6	39.0
Olympic plus urea			91.4 94.2	84.0 89.0		
	103.4	101.0	92.8	86.5	80.4	51.0

[a] The three percentages given in this column for each treatment and type of silt loam soil are for the top 3 inches, bottom 3 inches, and mean, respectively.

variation in microbial development in the check soil and there were no indications that the insecticides contributed markedly to increases or decreases of soil microörganisms. The results of insecticide analyses indicated that the microörganisms were contributing little if any to the insecticide changes. The high stimulation of molds and bacteria in the autoclaved soils was associated with the heat treatment which partially decomposes soil organic matter and makes it more available to the microörganisms. The microbial analyses are given in table 1.

Changes in pH in general were slight during the 11-month period and varied no more than would be expected with normal seasonal and climatic changes.

The results of the soil insecticide analyses (tables 2, 3, and 4) are summarized in table 5. Differences in recovery associated with soil type, autoclaving, or addition of urea, were not significant. At the eighth-month sampling there was no difference in the insecticide content, as determined by the bioassay and total chlorine methods, between the top and bottom 3 inches of the sample. Insecticide decline accordingly seemed not to be associated with leaching.

Although the objective of the study as to the factors contributing to differences in control of the garden sym-

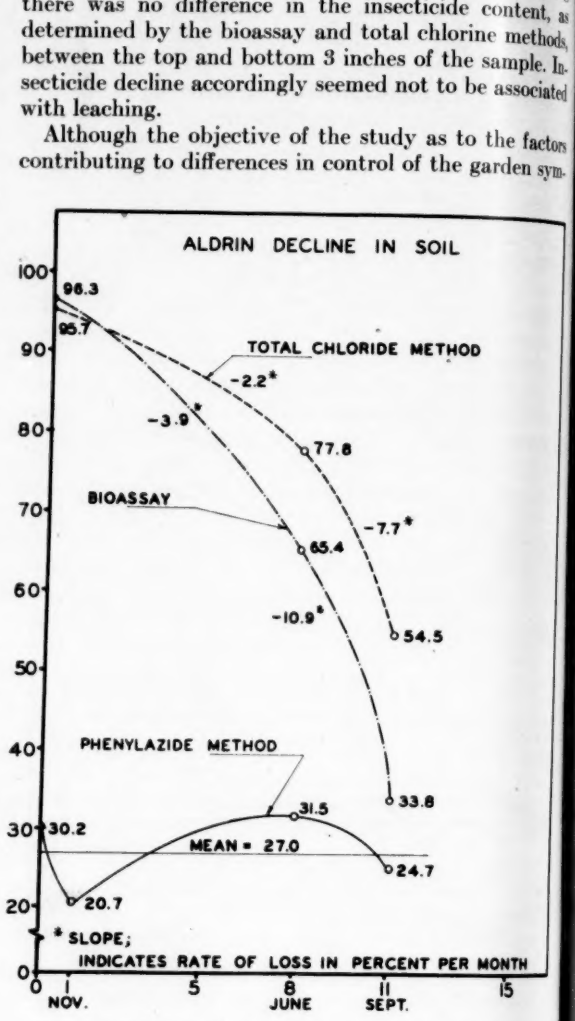

FIG. 1.—Aldrin decline in the soil as indicated by three different analytical methods.

Table 5.—Summary of soil analyses for insecticides.

Treatment and Rate (p.p.m.)	Analytical Method Used	Interval Between Treatment and Sampling							
		0 Days		One Month		Eight Months		Eleven Months	
		Mean	Standard Error of Mean	Mean	Standard Error of Mean	Mean	Standard Error of Mean	Mean	Standard Error of Mean
Aldrin 5.4	Total Chlorine	95.7	3.30	—	—	77.8	0.98	54.5	9.92
	Bioassay	96.3	2.69	—	—	65.4	3.10	33.8	3.23
	Phenylazide	30.2	8.32	20.7	3.18	31.5	3.55	24.7	2.84
Dieldrin 5.0	Total Chlorine	94.3	2.25	—	—	88.0	1.23	42.9	9.09
	Bioassay	93.2	2.39	—	—	78.6	1.32	31.8	2.24
	Phenylazide	66.0	7.23	78.0	6.88	78.7	7.22	83.3	3.44
Heptachlor 5.0	Total Chlorine	103.4	1.79	—	—	93.6	1.73	76.2	11.34
	Bioassay	102.8	1.17	—	—	51.4	2.93	37.8	2.60

phylid was not realized, certain differences in results due to different chemical methods are of interest.

Total chlorine and bioassay methods showed similar results with aldrin, dieldrin, and heptachlor; initial recoveries were high, followed by a rapid decline. The bioassay method also indicated that the decline in toxic compounds was almost equal for all three insecticides at 11 months. The recovery ranged from 32% to 38%.

With the exception of 0 time, the total chlorine method showed significantly higher insecticide content than the bioassay method. This seems to indicate that these compounds were breaking down to products that were less toxic than the original insecticides. These breakdown products still contained chlorine but apparently were sufficiently water insoluble to resist leaching.

The extraction procedure used for aldrin in the phenylazide method was only 50% efficient as shown by Bollen *et al.* (1958). This could explain the low recoveries obtained with aldrin. The results with the phenylazide method indicate little change in the aldrin and dieldrin content of the soil. No explanation can be given for this discrepancy between the specific and nonspecific methods.

The slopes of the curves obtained by total chlorine and by bioassay methods (fig. 1, 2, and 3) indicate that insecticide decline in the soil does not depend on the half-life of the toxicant. Since the rate of change is most rapid during the summer months, soil temperatures may be a contributing factor. The higher temperature in summer may either accelerate the breakdown of the material, or it may cause a loss through volatilization. The possibility of insecticide decline through volatilization is reduced by the fact that the soil was not disturbed during the experiment. That the temperature is an involved factor was substantiated by tests made by the Velsicol Corporation. Their analyses by a specific chemical method showed almost complete loss of heptachlor within 60 days after being mixed with the Chehalis and Olympic soils and placed in an oven at 50° C.

A comparison of the physical and chemical composition of the Chehalis and Olympic silt loams (table 6) shows only slight differences. Calcium and magnesium were present in a much higher concentration and in a narrower ratio in the Chehalis soil. This soil was also appreciably less acid as shown by pH and by the lime requirement. Since these two soils did not differ significantly in the rate of insecticide decline in the soil, it was concluded that these soil differences were of little importance in effecting insecticide change. The possible influence of metal ions, such as iron and zinc, was not investigated. Qualitative studies on the organic matter of different soil types might reveal some important influencing factors. Perhaps the most important field for further investigation lies in the determination of insecticide behavior in the presence of certain isolated mineral components of the soil.

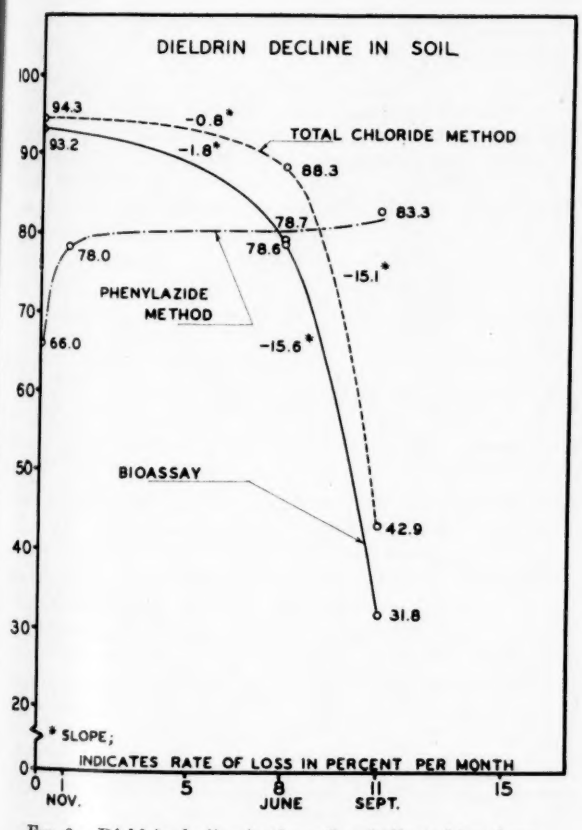

FIG. 2.—Dieldrin decline in the soil as indicated by three different analytical methods.

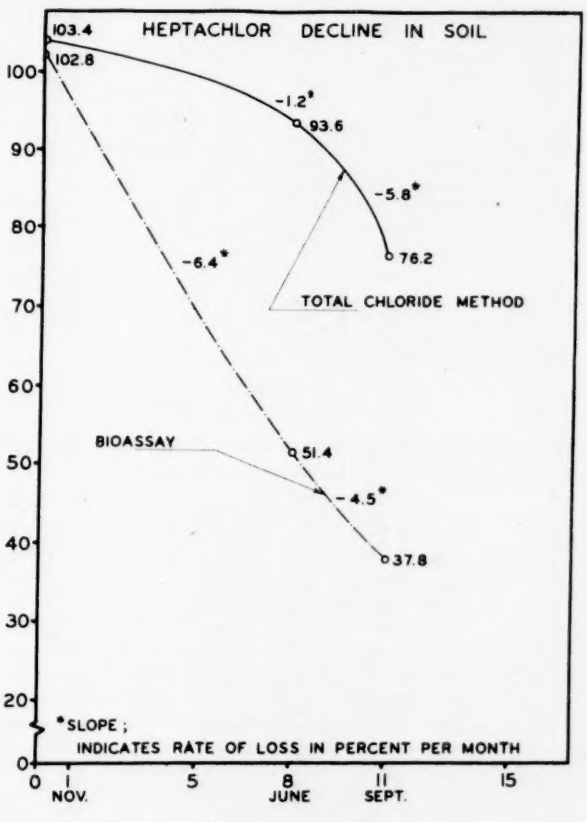

FIG. 3.—Heptachlor decline in the soil as indicated by two different analytical methods.

Table 6.—Results of physical, chemical and microbial analyses of Chehalis and Olympic silt loam soils.[a]

	Chehalis Silt Loam	Olympic Silt Loam
Mechanical analyses		
Fine gravel %	—	0.46
Coarse sand %	0.04	1.24
Medium sand %	0.05	1.82
Fine sand %	1.22	3.41
Very fine sand %	8.57	6.34
Silt %	69.94	68.60
Clay %	20.18	18.14
Texture	silt loam	silt loam
Moisture capacity %	53.7	38.3
Moisture equivalent %	28.88	23.54
Microbial analyses		
Bacteria (millions)	6.1	5.2
Streptomyces (%)	28.0	33.0
Molds (thousands)	126.0	19.0
Penicilla (%)	99.0	85.0
Mucors (%)	0.0	8.0
Chemical analyses		
Total carbons (%)	1.38	1.54
Organic matter (%)	2.38	2.65
Total nitrogen (%)	0.14	0.14
C/N ratio	9.85	11.00
Ammonium nitrogen p.p.m.	180.0	140.0
Nitrite nitrogen p.p.m.	0.1	0.1
Nitrate nitrogen p.p.m.	44.0	43.0
Sulfate sulfur p.p.m.	16.0	11.0
Phosphorus p.p.m.	23.0	29.0
Potassium p.p.m.	129.0	192.0
Calcium p.p.m.	2,405.0	1,183.0
Magnesium p.p.m.	1,760.0	390.0
Boron p.p.m.	1.6	1.3
NH_4-fixation capacity meq/100 gm.	2.7	3.7
Cation exchange capacity meq/100 gm.	28.3	14.0
pH (1:5 aqueous suspension)	6.6	5.9
Lime requirement (tons/acre)	1.5	2.5

[a] Water free basis.

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Fate of P^{32}-Labeled Bayer 21/199 in the White Rat[1]

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Abstract

The fate of Bayer 21/199, *O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate, has been studied by administering the P^{32}-labeled compound orally to white rats at an average dosage of 20 mg. per kg. of body weight. The compound was rapidly metabolized and excreted. About 78% of the radioactivity of the original dose was excreted via the urine within 24 hours. Paper chromatographic analyses indicated that none of the radioactivity in the urine was Bayer 21/199 but was associated with more polar compounds. Lesser amounts of radioactivity were found in the feces, bile, lymph, and blood. Among samples of various tissues taken 24 hours after dosage, small but significant amounts of radioactivity were found in bone, liver, and kidney.

Bayer 21/199, *O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate, has shown some systemic activity against the common cattle grub, *Hypoderma lineatum* (De Vill.) (Roth & Eddy 1955, Brundrett *et al.* 1957). Extensive work has been done on the fate of P^{32}-labeled Bayer 21/199 when applied dermally and orally to cattle (Lindquist 1957). The present study was undertaken to study the fate of P^{32}-labeled Bayer 21/199 in the white rat.

Materials and Methods.—The P^{32}-labeled Bayer

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21/199[3] had a specific activity of approximately 0.9 millicurie per gram. Male white rats (Sprague-Dawley strain), approximately 2 months old and weighing from 200 to 235 grams (average 225 grams), were used in these experiments. Each rat was dosed orally with 5 mg. of P^{32}-labeled Bayer 21/199 dissolved in 1 ml. of corn oil. This corresponded to a dosage of 20 mg. of Bayer 21/199 per kg. of rat body weight based upon an average weight of 225 grams.

The radioassay equipment used has been described previously (Schmidt & Dahm 1956), except for a Geiger-Mueller, dipping counter tube which was used to assay solutions of digested tissues and feces. Radioactive samples were counted long enough to attain a standard error of $\pm 5\%$ (Calvin *et al.* 1949). The surgical techniques employed for cannulating the bladder, common bile duct, and intestinal lymph duct have also been described previously (Burns 1957, Burns *et al.* 1957). The bladders of six rats (no. 1 through 6, table 1) and the intestinal lymphatic ducts of two of these rats (no. 5 & 6) were cannulated; the radioactive Bayer 21/199 was administered to the rats from two to six hours after surgery. Two other rats (no. 3 & 4, table 1) were dosed with the radioactive Bayer 21/199 after which the common bile duct of each rat was cannulated. The feces from all the rats were collected 4 and 6 days after dosage. Two additional rats (no. 7 & 8, table 1) were dosed as described previously and sacrificed 24 hours later for tissue distribution studies. Samples of brain, blood, muscle, fat, kidney, liver, and bone were removed and assayed radiometrically. The portions of tissue samples used for radioassay weighed from 0.5 to 1 gram.

Small volumes of urine, bile, lymph, and blood were pipetted directly onto aluminum planchets where they were dried and radioassayed. Samples of tissues and feces were digested with boiling, concentrated HNO_3 followed by the addition of 30% H_2O_2 and the resulting solutions assayed with the dipping counter tube. All radioassay data were corrected to the activity at the time of dosage. Descending, reversed-phase paper chromatography was used to assay the radioactive samples of urine, bile, and an acetone solution of P^{32}-labeled Bayer 21/199. Aliquots of these samples were applied to paper strips, prepared as described below, and the fluids evaporated with a stream of warm air. The mobile phase consisted of a 1:1 mixture of ethanol (95%) and water. The immobile phase was Dow Corning 550 Fluid, applied to 1-inch wide Whatman No. 1 filter paper by dipping the paper strips in a 5% (w/v) solution of the Dow Corning 550 Fluid in diethyl ether. The chromatograms were scanned radiometrically using equipment that has been described previously (Schmidt & Dahm 1956).

RESULTS AND DISCUSSION.—The average recoveries of radioactivity from P^{32}-labeled Bayer 21/199 administered orally to pairs of white rats are summarized in table 1. The average, accumulative radioactivity from the Bayer 21/199 administered that was excreted in the urine of rats 1 and 2 and secreted in the bile of rats 3 and 4 is shown in figure 1. About 78% of the radioactivity appeared as a water-soluble metabolite in the urine within 24 hours after dosage; only 2.5% appeared in the bile during this same period indicating a quite different metabolic pathway than for DDT (Burns *et al.* 1957). Paper chromatographic analyses of urine from rats 1 and 2, collected 4 and 9 hours after dosage, revealed a single peak of radioactivity at R_f 0.76 ± 0.04. The radioactive material in the urine was apparently more polar and quite unlike Bayer 21/199 in its response to this type of chromatographic separation. The fact that the other two pairs of rats (3

Table 1.—Recoveries of radioactivity from P^{32}-labeled Bayer 21/199 administered orally to white rats at the approximate rate of 20 mg. of Bayer 21/199 per kg. of body weight.

Sample[a]	Period of Collection (Hr.)	Rat No.	Radioactivity Calculated as Bayer 21/199	
			Per Ml. or Gm. (μg.)	Total Recovered (Per cent)
Urine	118	1	52	99[b]
	118	2	27	76
Feces	144	1	27	16[b]
	144	2	25	15
Urine	56	3	28	4.5
	80	4	70	11
Feces	96	3	99	9.5
	72	4	421	7.6
Bile	75	3	7.8	5.1
	68	4	6.1	2.7
Urine	40	5	218	43
	72	6	92	64
Feces	120	6	31	8.1
Lymph	40[c]	5	4.4	—
	72	6	2.5	—
Blood	4[c]	6	1.9	—
Brain[d]	24[c]	7	0.6	<0.1
		8	0.4	<0.1
Blood	24	7	1.0	—
		8	0.9	—
Muscle	24	7	0.9	—
		8	0.8	—
Fat	24	7	1.1	—
		8	0.8	—
Kidney[d]	24	7	5.2	0.2
		8	4.7	0.2
Liver[d]	24	7	11.0	2.3
		8	7.2	1.8
Bone	24	7	13.0	—
		8	9.8	—

[a] Samples of urine, bile, and lymph were obtained by cannulation of the bladder, common bile duct, and intestinal lymphatic duct, respectively.

[b] The fact that the sum of these percentages exceeds 100 was apparently due to a slight overdosage.

[c] The 40- and 72-hour periods shown for lymph and the 4-hour period shown for blood from rats 5 and 6 and the 24-hour period for all samples from rats 7 and 8 represent the time interval after dosage that these samples were taken.

[d] The mean weights of the brains, kidneys, and livers were 1.6, 2.3, and 11.3 gm., respectively.

[3] This material was synthesized in the laboratories of Farbenfabriken Bayer, Leverkusen, Germany and obtained through R. J. Geary of Vero Beach Laboratories, Inc., Vero Beach, Fla.

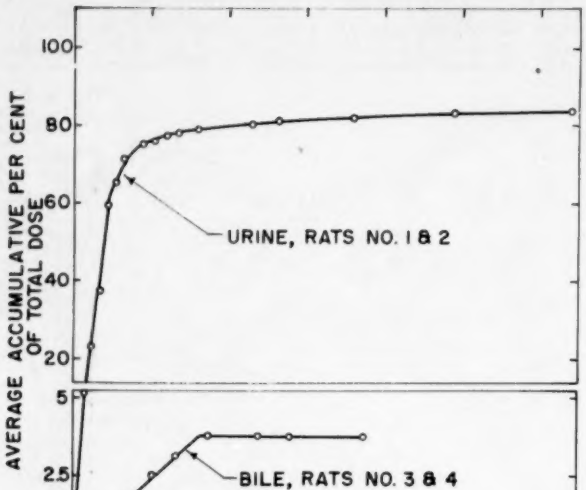

FIG. 1.—Average accumulative per cent of total dose, based on radioactivity, excreted via cannulated urinary bladders and secreted via cannulated common bile ducts of two pairs of rats following oral administration of approximately 20 mg. of P^{32}-labeled Bayer 21/199 per kg. of body weight. Note change in ordinate scale.

through 6, table 1) did not excrete as much urine as rats 1 and 2 appeared to be due to shock and slow post-operative recoveries concomitant with the cannulation of bile and lymph ducts.

Significant quantities of radioactivity (e.g. 7.6 to 16% of the total dose) from the labeled Bayer 21/199 were also excreted in the feces. Less radioactivity (e.g. 2.7 and 5.1% of the total dose) was recovered in the bile. Paper chromatographic analyses of bile from rats 3 and 4 showed a large single peak of radioactivity at R_f 0.60. It is possible that the radioactive material in the bile was similar or even identical to that found in the urine, since other non-radioactive compounds in the bile appeared to retard the movement of the radioactive material on the paper strip. The very small amounts of lymph collected made it difficult to measure accurately the amount of radioactivity in these samples.

Although relatively small amounts of radioactivity were found in the blood at collection intervals of 4 and 24 hours after dosage (rats 6, 7, & 8, table 1), it is probably significant that the greater amount of radioactivity was present at the shorter interval. This would correspond to a period when radioactivity was being excreted rapidly in the urine (cf. fig. 1). The bone, liver, and kidney samples from rats 7 and 8 (table 1) contained small amounts of radioactivity. These findings, combined with the analytical information from the urine, feces, and bile, indicate that the P^{32}-labeled Bayer 21/199 was rapidly broken down by the rat. The majority of the radioactivity was excreted in the urine and feces and some P^{32} was deposited in the bones.

Infrared[4] and ultraviolet spectra of technical grade and the P^{32}-labeled Bayer 21/199 indicated similar chemical composition and purity. When the radioactive sample of Bayer 21/199 was subjected to paper chromatographic analysis, a single peak of radioactivity (R_f 0.37 ± 0.02) was detected, indicating a high degree of radiochemical purity.

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[4]The infrared spectra were obtained through the courtesy of the Institute for Atomic Research, Iowa State College.

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Laboratory Evaluation of Insecticide-Treated Soils against the Western Subterranean Termite[1,2]

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ABSTRACT

The toxicity to the western subterranean termite (*Reticulitermes hesperus* Banks) of thin layers of damp, fine sand treated with seven insecticides was based on the period required for 50% immobility (MPI) or 50% mortality (MLP).

The relative effectiveness of the insecticides was different when MPI was used as the criterion than when MLP was used. With the former as a criterion, lindane was the most effective insecticide at all concentrations. However, when MLP was used as the criterion, pentachlorophenol was found to be the most effective insecticide at all concentrations down to 0.01% when it was no more effective than lindane.

Termites treated with pentachlorophenol, sodium pentachlorophenate and sodium arsenite died relatively soon after they were immobilized by the toxicants. Therefore there was less difference in the MPI and MLP values than was the case with the other insecticides used in these experiments.

The insecticides most adversely affected by successive dilutions were sodium arsenite, sodium pentachlorophenate and pentachlorophenol, and those least adversely affected were lindane, dieldrin and chlordane. When 1% concentrations of insecticide emulsions or solutions were applied, the MPI's and MLP's for insecticides in soil, 17 months after treatment, were generally approximately in the same ratio of relative effectiveness as they were for insecticides in freshly treated fine sand. However, this was not found to be the case with sodium arsenite, for it is a stomach poison, and fine sand is not ingested by the termites as readily as soil. Therefore results with sodium arsenite are relatively inferior when fine sand is the poisoned medium.

When the toxicity to termites of the upper half-inch of soil was compared with that of the inch of soil below, it was found to be greater with all insecticides except lindane. Emulsions were generally more adversely affected by passing through soil than were kerosene and water solutions. This was presumably due to the sieving out of the emulsified droplets of insecticide and solvent from the dilute emulsion. DDT was the most adversely affected among the emulsions, probably because (1) it was the most quick-breaking of the emulsions and (2) it is more adversely affected by dilution than lindane, dieldrin and chlordane. Sodium pentachlorophenate was the most adversely affected among the solutions.

Soil treated for 11 months with DDT, chlordane, dieldrin and lindane and placed on colonies of 50 termites in damp sand in glass test tubes was found to be an effective barrier against the usual upward movement of the termites. However, dieldrin was the only insecticide that had a significant toxic effect on the termites below the treated soil barrier.

Laboratory evaluations of insecticide-treated soils were made by Hetrick (1952, 1957). In these experiments, weighed amounts of insecticides were mixed with weighed amounts of moist, sandy soil and the treated soils were kept in 1-gallon glass battery jars under a building. A folded and moistened paper towel was placed on top of the soil in each battery jar. At intervals of about 2 months and extending over a 10-year period, 10 large workers of the eastern subterranean termite, *Reticulitermes flavipes* (Kollar), were placed on the damp paper towels and allowed to burrow into the moistened soil below. The period required for 100 per cent knockdown was determined. Lindane has remained effective at dilutions as great as 1 to 100,000 for 10 years and dieldrin, aldrin and heptachlor have remained effective at such dilutions for the 7-year period they have been tested. Other insecticides also showed prolonged residual activity, but at higher dosages.

Ebeling & Pence (1956) applied insecticide emulsions at 1% concentration of toxicant to "Yolo Clay Loam" soil in 4-ounce jelly jars. The soil had been sieved through regular fly screen (10 mesh). The dilute emulsions were applied at a dosage equivalent to 1 gallon to 5 square feet of soil surface. The objective was to test treated soil with a concentration of toxicant similar to that in soil that might be taken from the field after treatment in the generally accepted manner. Aliquot samples of the treated soils were placed in small vials and dampened. Ten worker western subterranean termites (*Reticulitermes hesperus* Banks) were placed in each of three vials of soil. The period for 50% mortality was determined, based on the period required for complete cessation of activity of the appendages. With the exception of lindane, the insecticides found by Hetrick (1957) to have the longest residual effectiveness at low concentrations in the soil were not the ones with the highest initial toxicity at 1% concentration. The marked differences in the initial and long-term performances of the various insecticides did not appear to be related to any differences in longevity of residues, at least as based on the performance of surface residues as revealed by extensive experience in agriculture. The experiments reported in the present paper were made in an attempt to ascertain what other factors might be involved.

TECHNIQUE.—When termites were placed in moistened soil in small vials (Ebeling & Pence 1956), those in the untreated soils began to die before mortality was complete in some of the less effective treated soils. Termites could not be kept alive long enough in the check lots. Another difficulty was that, although subterranean termites have a tendency to burrow next to the glass walls of the vials, where they can be easily seen, nevertheless a certain percentage are at a sufficient distance from the glass to result in their being hidden from view.

A technique was developed that had the advantage of keeping the termites alive longer and of preventing them from burrowing out of sight. Two circular disks of blotting paper were placed at the bottom of a stender dish with an inner diameter of 1 inch. On the top blotter was sprinkled 1 gram of insecticide-treated soil. Seven drops of water were added, moistening both the soil and the

[1] *Reticulitermes hesperus* Banks.

[2] Accepted for publication October 21, 1957.

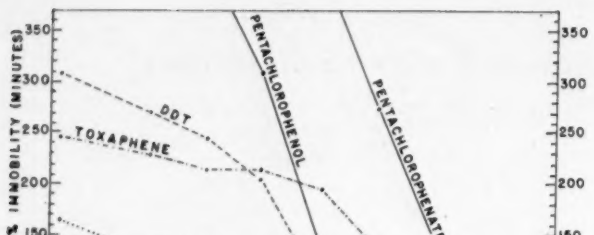

FIG. 1.—Relation of concentration of insecticide to toxicity to subterranean termites on treated sand, based on period for 50% immobility (MPI).

blotters below, and the stender dish was then covered with the usual glass top. The blotters appeared to serve as a regulatory mechanism, withdrawing moisture from the soil when the latter was excessively wet and giving up some moisture to the soil when it was too dry. Termites could be kept on untreated soils for at least a week without appreciable mortality.

Twenty termites in each of three stender dishes were used for each insecticide and concentration. Temperatures ranged from approximately 70° to 75° F. throughout the course of the experiments.

In contrast with the earlier experiments reported by Ebeling & Pence (1956) the period required for 50% immobility (MPI) was determined as well as the period for 50% mortality (MLP). A termite was considered to be immobile when its powers of locomotion were lost, even though the legs and other appendages might continue to be active for many hours or even for days.

It was believed that, since with the insecticides used in the experiments mortality always followed any appreciable paralysis, the period required to immobilize the termites might be of greater practical significance than the period required to kill them. If the termites were not paralyzed sufficiently rapidly, it is conceivable that they might eventually be able to burrow through the treated soil, even though large numbers might die in the process. Subsequently the termites might be able to pass through the relatively thin barrier of treated soil with so little contact with the insecticide that they might not be appreciably affected by it.

EXPERIMENTS WITH FRESHLY TREATED SAND.—For the present experiment a very fine sand was used, instead of soil, for the treated substrate. All of the sand passed through a 50-mesh screen. The sand was placed in $\frac{3}{4}$ inch $\times 3$ inch vials so as to be within a half-inch of the top. Then 2 ml. of insecticide emulsion or solution was poured into the vial and the sand was allowed to dry for several days. Using eight insecticides, the toxicity of the treated sand was determined, both with regard to the period required for 50% immobility and the period for 50% mortality, with concentrations of toxicant ranging from 0.01 to 4.0% (per cent referring to grams per 100 millimeters of water). All of the eight insecticides were tested simultaneously and with the same technique, so that the results could be directly comparable. The results are graphically presented in figures 1 and 2.

Figures 1 and 2 show a striking difference in the relative effectiveness of the eight insecticides when the MPI was utilized as a criterion (fig. 1) when compared with that which was found when the MLP was utilized (fig. 2).

At or near the concentrations of toxicant normally used for most insecticides, ranging from 0.5 to 1.0%, it is of interest to note the relatively high toxicity of lindane, when measured by the ability to rapidly immobilize the termites (fig. 1) and the relatively high toxicity of pentachlorophenol and sodium pentachlorophenate, when measured by the ability to effect a complete cessation of movements of the appendages (fig. 2). With the latter two compounds, as well as with sodium arsenite, the relative period between immobility and complete cessation of movement of the appendages ("mortality") is much less than with the other insecticides. This is again shown, with the 1% concentrations, in table 1.

A 4% concentration of sodium arsenite immobilized the termites so slowly (20 hours) that the curve for this insecticide could not be shown in figure 1. Nevertheless, based on the period required for complete cessation of movement of the appendages (MLP), sodium arsenite at 4% concentration was found to be among the more effective insecticides. However, sodium arsenite is at a disadvantage when sand is used in the experiment instead of orchard soil. Subterranean termites consume soil more readily than fine sand. Black-dyed clay loam soil can be seen entering the alimentary tract 5 minutes after termites are placed in the soil, whereas a period of $1\frac{1}{2}$ hours is required before a similar amount of sand is ingested. The alimentary tract eventually becomes filled with either soil or sand. However, more poison would be absorbed from the soil because more is ingested and the particles are smaller, resulting in a greater total area from which poison may be absorbed.

Another characteristic demonstrated by figures 1 and 2 is the great difference in the effect of dilution on the effectiveness of the different insecticides. A 400-fold decrease

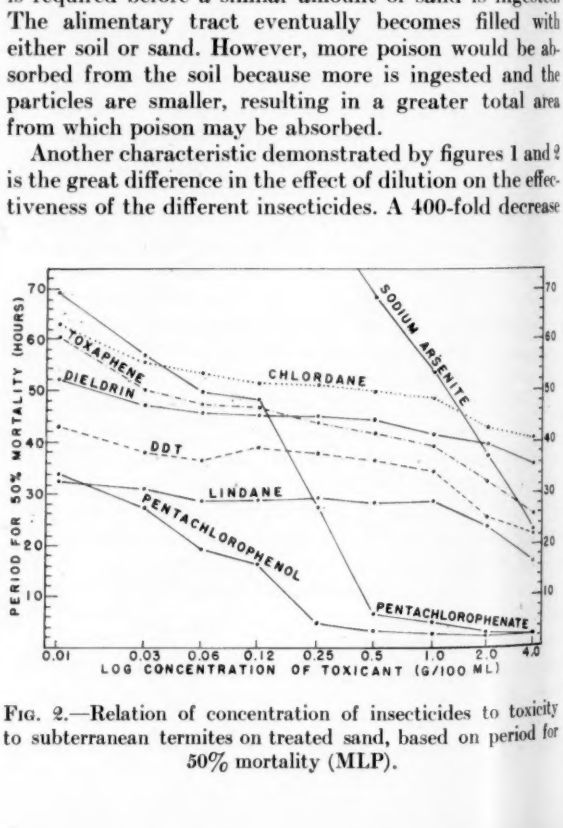

FIG. 2.—Relation of concentration of insecticides to toxicity to subterranean termites on treated sand, based on period for 50% mortality (MLP).

Table 1.—Period required for 50% immobility (MPI) and for 50% mortality (MLP) of subterranean termites placed on insecticide-treated sand.[a]

INSECTICIDE	MPI (MINUTES)	MLP (HOURS)
Lindane	8±0.6	29 ±2.5
DDT	59±2.1	35 ±2.6
Dieldrin	70±3.4	41 ±3.4
Chlordane	82±4.1	49 ±4.8
Pentachlorophenol[b]	88±4.6	2.5±0.1
Toxaphene	94±5.9	40 ±5.5
Sodium pentachlorophenate	147±8.0	5.1±0.2
Sodium arsenite	>350	54 ±7.2

[a] Emulsions or solutions containing 1% of toxicant were applied to very fine sand at a dosage equivalent to 2 gallons to 10 square feet of surface. The termites were placed on the treated sand as soon as it was dry.
[b] The insecticide was applied as a kerosene solution.

in concentration (from 4.0 to 0.01%) had remarkably little effect on the toxicity of lindane, dieldrin and chlordane, based either on MPI (fig. 1) or MLP (fig. 2). DDT and toxaphene were considerably more affected by dilution, particularly when based on MPI (fig. 1). Although DDT was more toxic than dieldrin and chlordane at 1%, it was less toxic than either of these insecticides at concentrations below 0.25%. As figures 1 and 2 show, pentachlorophenol, sodium pentachlorophenate and sodium arsenite were the insecticides most seriously affected by dilution of the toxicant.

EXPERIMENTS WITH YOLO CLAY LOAM 17 MONTHS AFTER TREATMENT.—Soil in 4-ounce jelly jars, that had been treated with 1% concentrations of insecticides as previously described, was used in the present experiment. This soil had been treated about 17 months previously, on September 22, 1955, and was from the same series as used in an earlier experiment (Ebeling & Pence 1956). Termites were placed on thoroughly mixed aliquot samples of the treated soils in stender dishes as described above. The MPI's and MLP's for the 16 insecticides are shown in table 2.

When comparing tables 1 and 2, it may be noted that particularly the MPI's are much higher in the latter, possibly indicating approximately the loss in the toxicity of the insecticides over the 17-month period. The exception is sodium arsenite, which, as stated above, is at a disadvantage when tests are made in sand, which was the medium used in the tests shown in table 1.

Although pentachlorophenol was greatly superior to sodium pentachlorophenate in table 1, there was no significant difference between these two compounds indicated by the data in table 2. This tends to confirm the belief held by some authorities that the sodium salt of pentachlorophenol changes to the parent compound over a period of time in the soil.

DDT, chlordane, and toxaphene were applied in kerosene as well as in water. The MPI's were higher when kerosene was used as the carrier, but the MLP's were lower. Since the samples of soil were taken from the first inch of soil, the aqueous emulsions were favored; for the dilution gradient, with increasing depth, is greater with emulsions than with solutions, as will be shown below.

TOXICITY OF TREATED SOIL AT DIFFERENT DEPTHS.—An experiment was made to compare the toxicity of the

Table 2.—MPI's and MLP's of subterranean termites placed on soil that had been treated[a] 17 months previously with 1% concentrations of various insecticides.

INSECTICIDE	MPI (MINUTES)	MLP (HOURS)
DDT	122± 3.1	51±3.2
DDT[b]	155±10.3	39±2.1
Chlordane	287±11.7	60±3.5
Chlordane[b]	383±37.8	48±2.1
Toxaphene	282±23.4	55±1.7
Toxaphene[b]	500±26.6	42±2.1
Heptachlor	120± 2.7	72±2.6
Lindane	30± 1.8	34±2.4
Aldrin	128± 6.9	75±2.7
Dieldrin	163± 3.6	62±2.6
Parathion	62± 3.3	41±1.0
Malathion	51± 3.2	48±1.2
Diazinon	83± 2.1	42±2.1
Pentachlorophenol[b]	287± 9.0	6±0.3
Sodium pentachlorophenate	240±15.4	6±0.3
Sodium arsenite	167± 3.8	5±0.3

[a] Emulsions or solutions containing 1% of toxicant were applied to soil in 4-oz. jelly jars at a dosage equivalent to 2 gallons to 10 square feet of surface. Treatments were made on September 22, 1955 and termites were placed on the treated soils on February 27, 1957.
[b] The insecticide was applied as a kerosene solution.

first half-inch layer of treated soil with the toxicity of the next inch layer below it. Aliquot samples of soil from these two strata were tested according to the stender dish technique described above. The soils were taken from lots that were treated about 16 months previously. Results of this experiment are shown in table 3.

Table 3 shows that the emulsifiable concentrates of DDT, chlordane, and dieldrin were considerably diluted by the time they had passed through the first half-inch of soil. Apparently even the very small globules of an emulsifiable concentrate are to a large extent strained out by the soil particles. The ratio of effectiveness between the upper and lower layer of soils was greatest with DDT. The DDT emulsion was the most quick-breaking of the four used, and consequently sieving out of the globules would be expected to be the greatest with this formulation. Also DDT is most adversely affected by dilution, and thus effectiveness would decline with depth of soil at a greater rate than for dieldrin, chlordane, and lindane.

Table 3.—Toxicity of various insecticide-treated soils to termites 16 months after treatment as affected by the depth from which the soil sample was taken.[a]

INSECTICIDE	PERIOD REQUIRED FOR 50% IMMOBILITY (MINUTES)	
	Upper Layer	Lower Layer
DDT	79± 4.0	940± 42
DDT in kerosene	103± 4.3	178± 18
Chlordane	327±18	1400±153
Chlordane in kerosene	470±49	850± 82
Dieldrin	145± 5.2	310± 10
Lindane	25± 1.5	25± 1.5
Pentachlorophenol in kerosene	182±11	316± 14
Sodium pentachlorophenate	192±10	807± 76
Sodium arsenite	148± 7.3	188± 8.4

[a] The soils were treated on September 22, 1955 and the tests were made on January 18, 1957. The "upper layer" consisted of the first half-inch, and the "lower layer" consisted of the next inch, of soil.

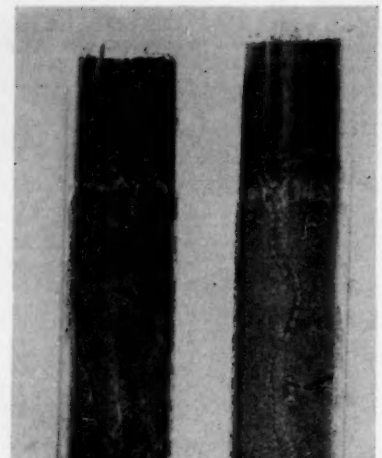

FIG. 3.—Termite colonies in moist fine sand in glass test tubes, with a 1-inch barrier of moistened insecticide-treated soil above. The termite tunnels reached the treated soil, but proceeded no further.

Lindane was not affected by soil depth, probably because it is so little affected by dilution. The small effect of depth of soil on sodium arsenite solution, as compared with sodium pentachlorophenate solution, is an interesting point concerning which no explanation is suggested.

It is understandable that the kerosene solution of DDT and chlordane should be less affected than the emulsifiable concentrates by the depth of the soil, for there were no discrete globules or particles to be strained out. However, even with these solutions, the soil samples taken below the first half-inch were considerably less toxic. Perhaps the soil became less saturated with toxic solution with increasing depth.

TESTING SOIL BARRIERS IN THE LABORATORY.—Subterranean termites can be kept alive and active in moist sand or soil in test tubes for a month or more, by wetting the soil by means of capillarity (Ebeling & Pence 1957). In the present experiments, 1-inch barriers of treated soil were placed on columns of termite-infested sand in 1×8 inch glass test tubes. Fifty worker termites were placed in each tube. Since the termites have a tendency to burrow next to the glass, their progress through the sand can be easily followed. An "applicator stick" was thrust through the column of sand, next to the glass, and allowed to extend 1 inch above the sand. This stick was for the purpose of providing wood upon which the termites could feed.

In order to prepare treated soils to cover these termite colonies, "Yolo Clay Loam" soil was sieved through a 10-mesh screen and placed in 10-inch-square pans to a depth of 2 inches. The soils were treated with emulsions or solutions of various insecticides at concentrations of 1% of toxicant. The amount of dilute emulsion or solution applied was equivalent to a gallon to 5 square feet of soil surface. The soils were kept at a temperature ranging from 70° to 80° F. Seventeen months later cross sections of soil were removed from the pans, thoroughly mixed, poured on top of the termite-infested sand in the glass tubes, lightly tamped to form a firm barrier of treated soil 1 inch in depth, and dampened (fig. 3). In this preliminary test only one tube was used for each insecticide.

In three tubes in which untreated soil had been placed on infested sand, the termites had penetrated through the inch of added soil when left overnight. The soil treated with 1% sodium arsenite was completely penetrated in 17 days. One month after the treated soil had been placed on the termite-infested sand, none of the remaining barriers had been completely penetrated. However, short tunnels made by the termites were found in the treated barriers. The insecticides used and depth of penetration by the termites were as follows: chlordane, 1 of $\frac{5}{8}$ inch along the applicator stick; chlordane in no. 2 fuel oil, 1 of $\frac{3}{8}$ inch and 2 of $\frac{1}{16}$ inch; lindane, 1 of $\frac{1}{16}$ inch; pentachlorophenol in no. 2 fuel oil, 3 of $\frac{1}{16}$ inch; sodium pentachlorophenate, in water, 1 of $\frac{1}{2}$ inch; and toxaphene, 2 of $\frac{3}{8}$ inch. However, in all cases the termites eventually discontinued their attempts to penetrate the soil. No penetration of termites through the treated soil occurred with DDT, DDT in no. 2 fuel oil; Diazinon, or dieldrin. In the tube on which the dieldrin-treated soil was placed, no termites survived more than 8 days, thus confirming earlier work (Ebeling & Pence 1956) that indicated that the vapors of dieldrin and lindane, at least when first applied, can penetrate downward or sideways through soil and kill termites beyond the treated area. Some termites survived for over a month in all the other tubes. In the tubes on which soil treated with DDT or pentachlorophenol was placed, some termites survived for more than 2 months. Even those in the tube with untreated soil placed over the colony had all died by that time.

The experiment was repeated again with soils treated 11 months previously with chlordane, DDT, dieldrin, and lindane, using five replicates for each insecticide. This time not even an abortive attempt was made by the termites to penetrate the treated soils. The number of days that passed before the last termite had died in each glass tube is shown in table 4. The only insecticide that significantly affected the rate of mortality of termites under the treated soil barriers was dieldrin. The average number of days required for the last termite to die in the five tubes was 10.4 for dieldrin and 31.3 for the untreated check. There was a great variation in the number of days of survival of termites in the five tubes in which the dieldrin-treated soil was placed. In the two tubes in which the termites survived as long as 16 and 21 days, they had concentrated in the lower parts of the tubes and had sealed themselves off from the galleries leading to the treated soil above.

Table 4.—Effect of insecticide-treated Yolo Clay loam soil, 11 months after treatment, on subterranean termites below the treated-soil barrier in glass test tubes.[a]

Insecticide	Period for 100% Mortality (Hours)					
	Trial No.					
	1	2	3	4	5	Average
Chlordane	27	18	29	36	30	28.0
DDT	22	29	30	44	36	32.2
Dieldrin	3	6	16	6	21	10.4
Lindane	22	29	29	33	24	27.4
Untreated	26	36	40	27	25	31.3
L.S.D. at the 5% level						8.9
at the 1% level						12.1

[a] The soils were treated with emulsions containing 1% of toxicant and kept in an unheated warehouse until the samples were taken.

Discussion.—The difference in the relative effectiveness between insecticides when first applied, as compared with their relative effectiveness after long periods in the soil, may be accounted for, at least in part, by the difference in the effect of dilution on the insecticides. The insecticides that have remained effective the longest at low concentrations in the laboratory experiments of Hetrick (1952, 1957) as well as in the field experiments of the United States Forest Service (Johnston 1956), are those that were found in the present investigation to be least affected by successive dilutions, regardless of their relative initial effectiveness. Presumably dilution would have an effect similar to that of decomposition or other factors that might diminish the effectiveness of insecticides in the soil.

Slight penetration of 17-month-old Yolo Clay Loam soil treated with certain insecticides was noted. However, this soil had been kept at much higher temperatures than those of soil occurring beneath buildings.

Dieldrin was the only insecticide found to have an effect on subterranean termites, below the treated area, 11 and 17 months after treatment. This was presumably through the effect of its vapors and was an unexpected phenomenon in view of the relatively low vapor pressure of dieldrin. However, the ultimate value of this apparent advantage of dieldrin can only be evaluated by means of long-term field experiments which are now in progress.

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The Phosphatase Activity of Susceptible and Resistant House Flies and German Cockroaches[1]

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Abstract

Differences in the phosphatase activity of susceptible and DDT-resistant house flies (*Musca domestica* L.) and susceptible and chlordane-resistant cockroaches (*Blattella germanica* (L.) and *Periplaneta americana* (L.)) have been measured. The phosphatase analysis was based upon the colorimetric determination of *beta*-naphthol liberated from sodium *beta*-naphtholphosphate.

Although significant differences were found in the acid and alkaline phosphatase activity between the sexes of both resistant and susceptible insects and also differences in the acid phosphatase activity between females of one DDT-resistant and one susceptible strain of insects, it was concluded that these differences were due to strain variations rather than to resistance.

A possible relationship of the alkaline phosphatase system to the detoxification mechanism in resistant flies was suggested by Rockstein & Inashima (1953), because of differences in alkaline phosphatase activity between only one DDT-resistant and one susceptible strain of house flies. Pratt & Babers (1953) studied the cholinesterase activity, oxygen consumption, and mortality responses of several strains of house flies and found that differences in oxygen consumption were statistically significant when one susceptible strain was compared with one resistant strain, but not when three susceptible strains were compared with three resistant ones. Several other investigators, working with only a few strains of insects, have also associated enzyme differences (dehydrochlorinase, cholinesterase, and succinoxidase) with resistance, only later to have their views questioned by others (Metcalf 1955, Crow 1957).

These investigations suggested that observed physiological differences between one resistant and one sus-

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[2] Now with: Quartermaster Research and Development U. S. Army Natick Mass.

ceptible strain of an insect are not necessarily related to resistance. Additional studies were therefore undertaken on the phosphatase activity of susceptible and resistant house flies (*Musca domestica* L.) and German cockroaches (*Blattella germanica* (L.)) and of susceptible American cockroaches (*Periplaneta americana* (L.)). American cockroaches have not developed appreciable resistance to insecticides.

TEST INSECTS.—The house flies were reared by the Peet-Grady method (Anon. 1951) and were fed a 5% aqueous glucose solution from adult emergence to death.

DDT-resistant flies (R-1), obtained from pupae originating from colonies at the Orlando, Fla., laboratory, were selected for DDT resistance for more than 200 generations. Pyrethrum-resistant flies (R-2) were from a culture obtained from the Army Chemical Center at Edgewood, Md.,[3] and had acquired an eight-fold tolerance to pyrethrum.

Susceptible strains of house flies (S-1, S-2, and S-3) were from the 1948 National Association of Insecticide and Disinfectant Manufacturers' stock, but each strain was reared by a different technique and had been inbred several years.

The cockroaches, German and American, were reared according to the procedure of Piquett & Fales (1952). They were fed dog biscuits and water. These dog biscuits were found free of phosphatase activity on analysis. Carbon dioxide was used as the anesthetic to facilitate the handling of the cockroaches.

The resistant German cockroaches were from a field-collected strain highly resistant to chlordane and moderately resistant to certain other insecticides (Heal, *et al.* 1953). The susceptible cockroaches were from strains maintained at Beltsville over a period of several years.

MATERIALS.—*Substrate.*—Sodium *beta*-naphtholphosphate, 20 mg., was diluted to 100 ml. with water to give a concentration of 0.0008 *M*.

Veronal Buffer (pH 7.5).—Approximately 950 ml. of 0.1 *M* sodium diethylbarbiturate was adjusted to the desired pH with 0.1 *M* hydrochloric acid and diluted to 1 liter. The mixture was filtered just before use.

Acetate Buffer (pH 4.8).—Approximately 500 ml. of 0.2 *M* acetic acid was diluted with 0.2 *M* sodium acetate to the desired pH.

Coupling Agent (Tetrazotized Diorthoanisidine (Du Pont's Naphthanil Diazo Blue B)).—The dye was prepared in a concentration of 4 mg. per milliliter in ice water just before use. Approximately 80 mg. of dye was mixed in 20 ml. of cold water, filtered, and kept in an ice bath while in use.

Glass Beads with DDT.—Glass beads 15 to 60 microns in diameter, 5 grams, were thoroughly mixed with 50 mg. of recrystallized DDT and 20 ml. of redistilled acetone, and the acetone was then evaporated. About 101 mg. of beads contained 1 mg. of DDT (Sternburg *et al.* 1954).

Enzyme Source.—House flies (usually four) 2 or 3 days old were separated by sex and pulverized in 10 ml. of distilled water. The mixture was centrifuged 4 minutes, and the centrifugate filtered through a porous, fluted filter paper. A 1-ml. aliquot of the filtrate was used immediately in the tests on alkaline and 0.2 ml. in those on acid phosphatase activity.

Adult German cockroaches (usually one to four) and the digestive tract of the American cockroach were treated in the same way.

PROCEDURE.—A modification of the procedure of Seligman *et al.* (1951), which is based upon the colorimetric determination of *beta*-naphthol liberated from sodium *beta*-naphtholphosphate, was used for the determination of phosphatase activity. The *beta*-naphthol in the range 300 to 900 mμ had a maximum absorption at 540 mμ. Experience indicated that consistent determinations could be obtained within a pH range of 7.4 to 7.7 or 4.8 to 5.0 at a *beta*-naphtholphosphate concentration of 200 micrograms per milliliter. Therefore, both the acid and alkaline phosphatase activities were determined.

Table 1.—Phosphatase activity in several strains of house flies and cockroaches.

STRAIN	AGE (DAYS)	WEIGHT OF INSECTS (MILLIGRAMS)	BETA-NAPHTHOL LIBERATED PER MILLIGRAM OF INSECT (MICROGRAMS)	
			Acid	Alkaline
Female house flies				
S-1	2	29.2	6.0±0.3	1.1±0.1
S-2	2	26.5	5.7±0.3	1.5±0.1
S-3	3	21.7	5.5±0.3	1.4±0.1
R-1	3	22.8	7.2±0.3	1.3±0.1
R-2	2	13.0	5.5±0.3	2.1±0.1
Male house flies				
S-1	2	18.7	4.8±0.3	1.8±0.1
S-2	2	17.2	3.8±0.3	1.6±0.1
S-3	3	17.0	3.1±0.3	1.0±0.1
R-1	3	19.0	3.1±0.3	0.9±0.1
R-2	2	11.4	4.3±0.3	1.7±0.1
Female German cockroaches				
S	Adult	108.0	15±1.9	—[a]
R	Adult	114.0	14±1.9	—
Male German cockroaches				
S	Adult	52.9	14±1.9	—
R	Adult	54.1	15±1.9	—
Digestive tracts of American cockroaches				
S Male	Adult	—	610±136	40±14
Female	Adult	—	1050±136	70±14

[a] Values too low to be measured by method.

The appropriate buffer solution, 75 ml., was mixed with 75 ml. of sodium *beta*-naphtholphosphate solution. A 5-ml. aliquot of the resulting solution was placed in a 20-ml. test tube containing an appropriate quantity of enzyme solution (usually 0.2 to 1 ml.) and incubated for 1 hour at 37° C. For determination of acid phosphatase activity, 0.2 ml. of 20% sodium carbonate was then added because the dye acts only in an alkaline system. After the incubation, 1 ml. of the coupling agent was immediately added, and the solution was shaken and allowed to stand 3 minutes. Then 1 ml. of 40% trichloroacetic acid was added and the mixture shaken with 10 ml. of ethyl acetate. The ethyl acetate extract was centrifuged and its percentage transmittance read at 540 mμ.

The procedure was similar for determining the activity in the presence of DDT, except that the appropriate

[3] Furnished by F. S. Snyder.

quantity of DDT beads was added to the 20-ml. test tubes with shaking prior to incubating.

RESULTS.—The results of these determinations are given in table 1. The data for the house fly are the averages for 6 generations and 15 analytical determinations; those for the German cockroach of 21 determinations on the same generation, and those for the American cockroach of 8 determinations on the same generation. The data were subjected to analyses of variance[4] as shown in table 2.

Rockstein and Inashima worked with mixtures of male and female house flies, but we separated our insects according to sex because sexual differences in phosphatase activity had been observed in house flies. Since a predominance of either sex would bias their results, it is difficult to compare their data with ours. Our investigation shows that the acid phosphatase activity of the female flies of strains S-1, S-2, S-3, and R-2 did not differ significantly, but in all these strains it was significantly different from that in strain R-1 at the 1% level. However, when the data on females of the five strains were compared collectively, these differences had only borderline significance at the 5% level. Similarly, the differences in alkaline activity were not significant when the five strains were compared collectively. Repeated investigations, even with variations in methodology, always showed, in acid phosphatase, differences only in the females. Because the male house fly is also resistant, resistance certainly cannot be explained in terms of females only. However, both normal and resistant house flies contain high levels of phosphatase.

Table 2.—Data subjected to analyses of variance.

		Micrograms of Beta-Naphthol Liberated per Milligram of Insect			
		Acid Activity		Alkaline Activity	
Strain	Sex	Amount	Differences Between Sexes or Strains	Amount	Differences Between Sexes or Strains
		House flies			
S-3	F	5.5	2.4*	1.4	0.5*
	M	3.1		0.95	
R-1	F	7.2	4.1*	1.3	0.4*
	M	3.1		0.93	
S-3	F	5.5	1.7*	1.4	0.1
R-1	F	7.2		1.3	
S-3	M	3.1	0	0.95	0
R-1	M	3.1		0.93	
		German cockroaches			
S	F	15	1	—	—
	M	14		—	—
R	F	14	1	—	—
	M	15		—	—
S	F	15	1	—	—
R	F	14		—	—
S	M	14	1	—	—
R	M	15		—	—

* Differences are significant at 1% level. L.S.D. (1%) 0.13 for acid and 0.31 for alkaline phosphatase.

Table 3.—Effect of addition of DDT to breis of susceptible and resistant female house flies on acid phosphatase activity.

	Micrograms of Beta-Naphthol Liberated per Milligram Insect		
Strain	No DDT	0.5 mg. DDT	1.0 mg. DDT
S-3	5.7	1.4	1.3
R-1	7.3	4.4	1.7

The data in table 3 show that DDT has a greater influence on the acid phosphatase activity of susceptible than of resistant female flies. It was not ascertained whether this influence is primary or secondary, but we suspect that it is a result, not a cause, of resistance. Therefore, it is concluded that observed differences in phosphatase activity between one resistant and one susceptible strain of house flies are insufficient evidence to relate the phosphatase enzyme system to resistance.

No important differences in phosphatase activity between the sexes were observed in German cockroaches, nor were any observed when susceptible and resistant cockroaches were compared.

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[4] An analysis of variance was made by J. U. McGuire, formerly of Biometrical Services, Agricultural Research Service, U.S.D.A.

Soil Properties and Factors Influencing Aldrin-Dieldrin Recovery and Transformation[1]

W. B. Bollen,[2] J. E. Roberts,[3] and H. E. Morrison[4]

Abstract

Variations in insecticidal effectiveness of aldrin and dieldrin in two different soils led to investigation of the persistence and possible transformation under field conditions. After 21 months, nearly one-half of the 5.4 p.p.m. of aldrin originally applied was lost, although a significant amount was recovered as dieldrin. Dieldrin loss from dieldrin-treated plots was less than aldrin loss from aldrin-treated plots, and no significant transformation to aldrin was detected. Microbial activity appeared to be unimportant in these changes.

Aldrin and dieldrin recoveries did not appear to correlate with buffer capacity, ammonium-binding power, cation exchange capacity, mechanical analysis, moisture tension, pH or chemical analyses of the soils. No single soil property appeared responsible for variations in effectiveness of the two insecticides. Properties found sufficiently different in the two soils to indicate that they may be concerned with this problem were their cation exchange capacities and their calcium and magnesium contents.

The phenylazide method used on extracts of 20-gram soil samples was sensitive to 0.2 p.p.m. of aldrin and 0.4 p.p.m. of dieldrin. The method for extraction of aldrin needs additional investigation. Successive extractions with the usual aldrin solvent, Skellysolve B, gave higher recovery than single extraction. The dieldrin solvent, Skellysolve B plus 25% acetone, gave greater recovery of aldrin from soil with a single extraction. It appears that none of the methods used in these and in previous studies gave a complete picture of what happens to aldrin and dieldrin after their contact with the soil.

The studies on insecticide decline described (Kiigemagi *et al.* 1958) yielded no suggestions to account for variations in insecticide performance in different soils. Since soils differ in pH, organic matter, texture and other physical and chemical properties, one or more of these factors could possible influence the recovery of soil insecticides. Accordingly, laboratory tests were carried on to determine if the changes could be associated with such factors as (1) physical and chemical properties of soils, (2) organic matter content, (3) microbial relationships, (4) methods of chemical extraction of insecticides from the soil and (5) insecticide transformations.

Procedure.—In addition to the two soils used in the preceding studies several other soil types were investigated. Only pure aldrin and dieldrin were used in the various supplemental studies, using recrystallized compounds instead of the dilute dusts previously employed. The phenylazide method of O'Donnell *et al.* (1952, 1954, 1955) was used for aldrin and dieldrin analysis. Other procedural details are described under each of the following experiments.

Physical Properties of Soils.—Mechanical analysis, soil moisture tension curves, and moisture equivalent of the soil samples were determined by the Soils Department, using the U. S. Department Agriculture pipette method (Kilmer & Alexander 1949), the pressure membrane and porous plate apparatus (Richards 1947, 1948), and the centrifuge method (Briggs & McLane 1907) respectively.

Chemical Analysis of Soils.—Total carbon was determined by dry combustion. Analyses for total nitrogen were made by the Kjeldahl-Hibbard method (Assoc. Official Agric. Chemists 1950). Nitrates were determined with phenoldisulfonic acid (Harper 1924) and nitrites with alpha-naphthylamine and sulfanilic acid (Amer. Publ. Health Assoc. 1955, p. 153). A turbidimetric procedure was used for sulfates (Amer. Publ. Health Assoc. 1955, p. 197). Ammonium nitrogen was determined by distillation with phosphate buffer at pH 7.4 (Shirkhande 1941).

Other chemical analyses presented in table 5 were made by the Soil Testing Laboratory (Alban 1954).

A comparison of the physical and chemical composition of the Chehalis and Olympic silt loams showed only few appreciable differences. (See Kiigemagi *et al.*, table 6, p. 204 this issue.) Calcium and magnesium were present in a much higher concentration and in a narrower ratio in the Chehalis soil. This soil was also appreciably less acid as shown by pH and by the lime requirements, and had approximately twice as much cation exchange capacity as the Olympic soil.

The Olympic silt loam was found to have a greater buffer capacity than the Chehalis soil toward acid. The buffer capacity of these soils and of Attaclay was determined by titrating 10 gram samples with 0.1 N NaOH and 0.1 N H_2SO_4. The amounts in gram equivalents required to change the pH value one unit were as follows:

	0.1 N Acid	*0.1 N Base*
Chehalis silt loam	0.08	0.05
Olympic silt loam	0.15	0.05
Attaclay	0.20	0.22

These differences in properties of the two soils suggest that inactivation or enhancement of insecticidal activity of aldrin and dieldrin, despite their water-insolubility, could be influenced by soil forces which function in adsorbing moisture, ions and molecules. Differences in inactivation of such insecticides may be related to types of clay minerals and to adsorption forces rather than to moisture *per se*.

Figure 1 compares the moisture tension curves of the two soils. This tension is an indication of the physical forces holding water within the soil. Such forces, particularly adsorption, may function in holding insecticides. Soils showing appreciably different moisture tensions

[1] Technical Paper No. 1078, Oregon Agricultural Experiment Station, Corvallis. Accepted for publication October 24, 1957.

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could be suspected of holding and possibly inactivating insecticides to different extents. The moisture content usually considered optimum for soil microbial activities is 50% of the water holding capacity. The corresponding moisture percentages and tensions are 26.9% and 0.55 atmosphere for the Chehalis soil, 19.2% and 0.70 atmosphere for the Olympic soil. These results correspond reasonably well with the mechanical analyses (table on p. 204 of this issue) when the better structure of the Olympic soil is considered. Since better insecticide activity in the Chehalis soil has been observed in the field, it seems possible that sorption is a factor which could be directly associated with binding the insecticides.

The Olympic soil also showed a slightly higher ammonium binding capacity as determined by the method of Moodie & Leggett (1955). This method is based on the recovery of ammonium nitrogen from an ammonium saturated sample. The ammonium remaining fixed in the soil after aerating the suspension made alkaline with 5% potassium carbonate is considered bound or fixed.

The capacity of a soil for binding ammonium is attributable to such minerals as montmorillonite and vermiculite which have an expandable lattice structure. Whether or not such minerals can bind aldrin or dieldrin requires further study. X-ray diffraction studies on these soils, made by J. A. Kittrick,[5] showed that illite was dominant in the collodial fractions of the Olympic soil. The illite content of the Chehalis soil was also high, and there were appreciable proportions of montmorillonite and vermiculite. Because of their expandable lattice structure these two minerals could possibly function in the sorption or fixing of aldrin or dieldrin. However, insecticide recovery from the two soils differed little. Moreover, the Chehalis soil appeared to promote greater insecticidal activity in the field. Hence the results of the diffraction studies and the ammonium binding capacity of the soils seem to offer no explanation for the differences in insecticidal behavior.

ORGANIC MATTER.—Since a high correlation between adsorption of CMU (p-chlorophenyl dimethyl-urea) and the per cent of soil organic matter was reported by Sherbourne & Freed (1954) this was considered a factor which might be explored. However, the Chehalis and Olympic silt loams were found to differ little in organic matter content, so this hardly could have constituted a quantitative factor with these soils. Samples of Chehalis silt loam, Gooch fine sandy loam and Palouse silt loam soils varying considerably in pH and organic matter were selected to explore possible influence of these factors. For comparative purposes Attaclay, commonly used as a diluent for insecticides, was included. It is essentially a pure clay, a hydrated aluminum-magnesium silicate with a particle size of 1 to 2 microns.

Solutions of aldrin and dieldrin in extraction solvent were mixed with Attaclay and these soils at the rate of 5 parts per million. After air-drying for 18 hours, the insecticides were extracted and aliquots of the extracts were analyzed by the phenylazide method as described in the previous paper (Kiigemagi *et al.* 1958). Results are tabulated in table 1.

Although the insecticides were extracted within 18 hours of mixing, there were extensive losses, amounting to 26 to 44% with aldrin and 2 to 32% for dieldrin. With each toxicant, the greatest loss was from the alkaline Gooch soil. There was apparent loss with Attaclay also, amounting to 50 and 6% for aldrin and dieldrin, respectively. These data (table 1) indicate no correlation of insecticide decline with pH or organic matter.

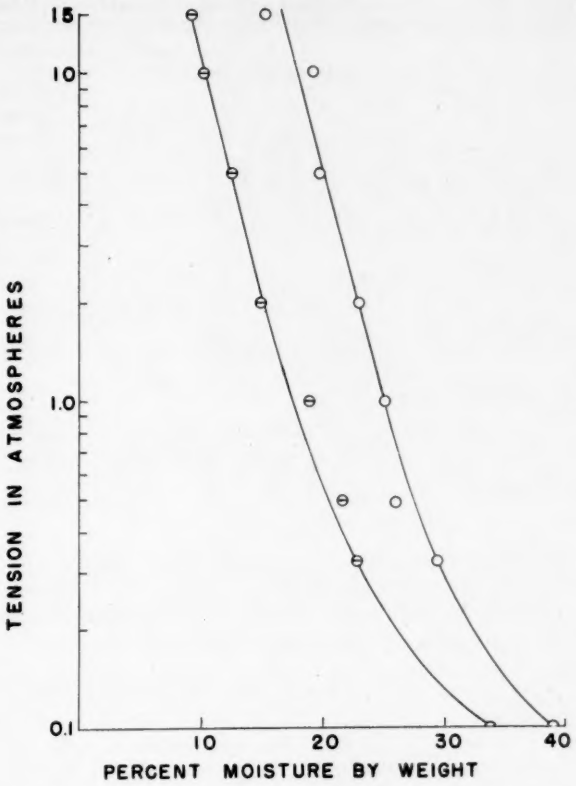

FIG. 1.—Moisture tension curves for Chehalis (-○-) and Olympic (⊖) silt loam soils.

In order to study further the possible influence of organic matter on the amount of insecticide recovered, samples of Attaclay (0.28% carbon) and Palouse silt loam soil (1.57% carbon) were heated for 3 hours at 300, 500 and 800 degrees C. After cooling to room temperatures aldrin and dieldrin were mixed at the rate of 5 parts per million in the Palouse soil and 20 parts per million in Attaclay. Mixtures of aldrin and dieldrin with unheated Palouse soil and Attaclay were used for comparison.

Extractions for analyses were made within 20 hours after insecticides were mixed with the respective materials. The results, given in table 2, show that the aldrin recovered in both Palouse soil and Attaclay increased as the heat treatments were increased. Dieldrin recoveries appeared to decrease as the heat intensity was increased on the Palouse silt loam. Dieldrin recoveries from Attaclay showed no definite trend. Whether or not the heat treatment effects were due to destruction of organic matter or to alteration of minerals in unknown. Malina *et al.* (1956) found that some chlorinated organic insecticides show marked decomposition on Attaclay and certain other

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Table 1.—Recovery of aldrin and dieldrin from different soils after 18 hours.[a]

Soil Type	Physical and Chemical Properties[b]						Insecticide Treatment			
	pH	Per Cent N	Per Cent C	C/N	Moisture Capacity	Organic Matter[c]	Moisture (Per Cent)	Toxicant	Added	Found	Apparent Loss (Per Cent)
Chehalis Silt Loam	5.7	0.12	0.67	5.6	66.0	2.77	17.3	Aldrin	5.0	3.7	26
								Dieldrin	5.0	4.5	10
Gooch Fine Sandy Loam	10.1	0.15	1.57	10.4	36.7	1.15	5.3	Aldrin	5.0	2.8	44
								Dieldrin	5.0	3.4	32
Palouse Silt Loam	6.6	0.13	1.61	12.2	57.2	2.70	23.6	Aldrin	5.0	3.0	40
								Dieldrin	5.0	4.9	2
Attaclay	7.3	0.00	0.28	—	216.0	0.00	5.9	Aldrin	5.0	2.5	50
								Dieldrin	5.0	4.7	6

[a] Phenylazide method of analyses, standard extractions. Sensitivity of determinations: 0.2 and 0.4 parts per million for aldrin and dieldrin, respectively.
[b] Water free basis.
[c] Calculated: Total carbon ×1.72.

mineral carriers, and that oxygen-containing chemicals such as diethylene glycol when used in formulations are effective in stabilizing heptachlor. The degrading influence of such carriers is associated with a high surface acidity.

INFLUENCE OF SOIL MICROBES.—To determine the influence of the soil microflora on aldrin and dieldrin and their possible interconversion a laboratory study was made using non-sterile, ethyline oxide-sterilized and autoclave-sterilized soils. The insecticides were added at five p.p.m. to 20-gram soil samples, water-free basis, in 50-ml. erlenmeyer flasks. Moisture was adjusted to 50% of the water holding capacity and the flasks were incubated at 28° C. Each sterilized soil was checked for sterility at the times of sampling and in all cases the nutrient agar plates poured with 1-gram samples were found to be sterile.

Table 2.—The effect of heat treatment of soil on recovery of aldrin and dieldrin.[a]

Soil	Heat Treatment[b]	Insecticide Treatment			
		Toxicant	Added	Found	Apparent Loss (Per Cent)
Palouse Silt Loam	None	Aldrin	5.0	3.0	40
		Dieldrin	5.0	4.9	10
	300	Aldrin	5.0	3.5	30
		Dieldrin	5.0	5.1	0
	500	Aldrin	5.0	3.9	22
		Dieldrin	5.0	4.3	14
	800	Aldrin	5.0	4.3	14
		Dieldrin	5.0	4.3	14
Attaclay	None	Aldrin	20.0	10.0	50
		Dieldrin	20.0	19.0	5
	300	Aldrin	20.0	13.0	35
		Dieldrin	20.0	10.0	50
	500	Aldrin	20.0	13.0	35
		Dieldrin	20.0	14.0	30
	800	Aldrin	20.0	15.0	25
		Dieldrin	20.0	20.0	0

[a] Phenylazide method of analyses, standard extractions. Sensitivity of method 0.2 and 0.4 parts per million for aldrin and dieldrin, respectively.
[b] Three hours at degrees centigrade indicated.

A preliminary experiment was made to determine if soil sterilization *per se*, through induced physical or chemical changes, would affect recovery of aldrin or dieldrin added after autoclaving or ethylene oxide treatment. Except for a higher recovery of dieldrin from the autoclaved soil, sterilization by either method had no significant influence on apparent losses (table 3).

For a more extended study replicate flasks were prepared for analysis at 1, 7, and 14 days. The extracts from aldrin-treated samples were analyzed for both aldrin and dieldrin and the extracts from dieldrin-treated samples were analyzed similarly. This procedure was designed to detect any non-microbial transformation of the toxicants. Table 4 shows the results.

Aldrin recoveries after 1 day in the Chehalis soil treated with aldrin were 60 to 62%. After 7 days they were from 76 to 84% and after 2 weeks 38 to 64%. Dieldrin in amounts above the sensitivity of the method was found in some of the aldrin treated soils. Soil treated with dieldrin showed no aldrin in the sterilized series; in the non-sterilized soil the 0.4 p.p.m. aldrin found at 1 and 14 days could be significant from the standpoint of transformation.

The data for sterile and non-sterile soils are sufficiently similar in values and trends to indicate that the presence

Table 3.—Recovery of aldrin and dieldrin from sterilized and non-sterile Chehalis silt loam soil after 20 hours.[a]

Method of Soil Sterilization	Toxicant	Amount (p.p.m.)		Apparent Loss (Per Cent)
		Added	Recovered	
None	Aldrin	5.0	3.7	26
	Dieldrin	5.0	4.5	10
Autoclaved 4 hours at 15 lbs. pressure and 121° C.	Aldrin	5.0	3.9	22
	Dieldrin	5.0	4.9	2
Ethylene oxide	Aldrin	5.0	4.0	20
	Dieldrin	5.0	4.5	10

[a] Phenylazide method of analyses, standard extractions. Sensitivity of determinations: 0.2 and 0.4 parts per million for aldrin and dieldrin, respectively.

Table 4.—Insecticide transformations in sterile and non-sterile soils.

Silt Loam Soil Type	Treatment		Incubation Period (Days)	Toxicant Recovered[a] (p.p.m.)	
	Method of Sterilizing	Toxicant (5 p.p.m.)		Aldrin	Dieldrin
Chehalis	None	Aldrin	1	3.6	0.2
			7	4.0	0.6
			14	2.7	0.0
Olympic	None	Aldrin	1	4.9	0.2
			7	3.8	0.5
			14	4.3	0.5
Chehalis	Autoclaved	Aldrin	1	3.0	0.0
			7	4.2	—
			14	3.2	0.0
Olympic	Autoclaved	Aldrin	1	2.5	0.4
			7	3.8	0.0
			14	4.0	0.0
Chehalis	Ethylene oxide	Aldrin	1	3.0	0.0
			7	3.9	0.0
			14	1.9	0.0
Olympic	Ethylene oxide	Aldrin	1	4.1	0.2
			7	3.8	0.4
			14	4.3	0.9
Chehalis	None	Dieldrin	1	0.4	5.0
			7	0.0	5.0
			14	0.4	2.2
Olympic	None	Dieldrin	1	0.4	4.2
			7	0.0	4.5
			14	0.7	5.6
Chehalis	Autoclaved	Dieldrin	1	0.0	4.4
			7	0.0	—
			14	0.0	3.8
Olympic	Autoclaved	Dieldrin	1	0.6	4.9
			7	0.0	4.2
			14	1.0	3.9
Chehalis	Ethylene oxide	Dieldrin	1	0.0	4.2
			7	0.0	4.2
			14	0.0	5.1
Olympic	Ethylene oxide	Dieldrin	1	0.5	4.4
			7	0.0	4.7
			14	0.2	5.0

[a] Sensitivity of method: 0.2 and 0.4 parts per million for aldrin and dieldrin, respectively.

of microörganisms was at most of minor importance in affecting recovery of aldrin or dieldrin. These insecticides appear not susceptible in the soil to a microbial attack that can result in their decomposition or appreciably alter their structure in a short time. That one autoclaved soil treated with dieldrin showed 1 p.p.m. aldrin and 4 p.p.m. dieldrin after 14 days suggests some action other than microbial in this instance of apparent transformation.

Results of the analyses after 1 day show that physical or chemical changes induced by soil sterilization, either by autoclaving or by ethylene oxide, do not significantly alter aldrin and dieldrin recoveries.

These results are in accord with those of Anderson *et al.* (1955) who found that applications of aldrin and dieldrin to California soils resulted in little increase in organic chloride content in the soil after 1 year However, after 2 years, with additional insecticide application, appreciable amounts of extractable organic chloride appeared, particularly when dieldrin was applied. Increases in organic chloride occurred also with aldrin-treated soils but to a lesser extent. This accumulation seems opposed to microbial decomposition of aldrin and dieldrin since the organic chloride insecticides would be expected to disappear more

Table 5.—Successive extractions for recoveries of aldrin and dieldrin 20 hours after mixing with Chehalis silt loam soil.[a]

Toxicant	Added (p.p.m.)	Recovered	(p.p.m.)	Per Cent Recovery[b]
Aldrin	5.0	First extraction	2.30	46.0
		Second extraction	.78	15.6
		Third extraction	.54	10.8
		Total	3.62	72.4
Dieldrin	5.0	First extraction	4.80	96.0
		Second extraction	.42	8.4
		Third extraction	0.00	0.0
		Total	5.22	104.4

[a] Phenylazide method of analyses; using Skellysolve B as extractant for aldrin and Skellysolve B plus acetone for dieldrin.
[b] Sensitivity of method: 0.2 and 0.4 parts per million for aldrin and dieldrin, respectively.

rapidly after prolonged exposure to the soil microflora, especially in view of the potential adaptivity which microörganisms exhibit.

STUDIES ON THE EXTRACTION PROCEDURES.—As the experimental work progressed, it became evident that aldrin recoveries from treated soil were always lower than those of dieldrin. This was true even when extractions were made within 1 day of mixing insecticides with the soil. Either physical or chemical changes were altering or sequestering the toxicants, or methods of extraction were faulty. Accordingly, tests were undertaken to evaluate the efficiency of the solvents and extraction methods as recommended by the Shell Chemical Company (O'Donnell *et al.* 1954). In these tests, it was demonstrated that the dieldrin solvent, Skellysolve B plus acetone, was more efficient for extracting aldrin from the soil than was Skellysolve B alone.

Tests were made with Chehalis silt loam soil which had been treated with aldrin and dieldrin at the rate of 5 parts per million. Three successive extractions for aldrin with the use of the Skellysolve B increased the aldrin recovery from 46% to 72% (table 5). In contrast to this, dieldrin recovery from an extraction with Skellysolve B plus 25% acetone was 96%. A second extraction increased this to 104%, while a third extraction yielded no more. Using single extractions on the Chehalis and Olympic soils treated with 5 p.p.m. aldrin the dieldrin solvent gave better recoveries than the aldrin solvent (table 6).

These tests show that although Skellysolve B is an excellent solvent for free aldrin, it seemingly does not readily contact the insecticide in the soil or overcome some competitive binding or protective powers. Inclusion of acetone makes a better solvent, probably because it effects better wetting, expecially if much moisture is present.

All aldrin recoveries up to this time had been determined by one extraction with Skellysolve B. Since this extraction procedure was demonstrated to be inadequate, probably previous results indicated much less aldrin than actually present in the soil. The mixed solvent was used for aldrin extraction in the sterile soil experiments and gave approximately 70% average recovery (tables 3 and 4). Previous recoveries of aldrin from all soils with Skelly-

Table 6.—Aldrin recovered with different solvents and single extraction 20 hours after mixing insecticide with soil.

Soil	P.p.m. Added	Recovered (p.p.m.)[a] With Skellysolve B	Recovered (p.p.m.)[a] With Skellysolve B plus 25% Acetone
Chehalis	5	3.9	4.3
Olympic	5	4.1	4.9

[a] Sensitivity of method: 0.2 and 0.4 parts per million for aldrin and dieldrin, respectively.

solve B as the recommended solvent average only 50%. Results obtained in the laboratories of Julius Hyman and Company (Shell Oil Company) on single extractions of soil samples submitted from a previous investigation (Fletcher & Bollen 1954), showed 26 to 50% recovery of aldrin added to Willamette clay loam soil.

ALDRIN-DIELDRIN TRANSFORMATION.—Probably the most significant indication from the foregoing studies was that transformations of aldrin to dieldrin and of dieldrin to aldrin apparently were taking place (table 4). These changes occurred in both sterile and non-sterile soil within relatively short time. The incubation period seems to be of little importance, since changes in both directions were detected after 1 day.

A final study on the insecticide transformations under field conditions was made as follows: 623 days after the insecticides were applied, five 100-gm. subsamples, each 6 inches deep, were drawn randonly from an 18×36 inch grid imposed on the aldrin and dieldrin cells of the Entomology field experiment (Kiigemagi *et al.* 1958). Each subsample was analyzed in duplicate for aldrin and dieldrin by the phenylazide method, using Skellysolve B plus 25% acetone as the extractant in each case. The results, presented in table 7, show (1) appreciable conversion of aldrin to dieldrin, (2) little conversion of dieldrin to aldrin, (3) approximately 50% unaccountable loss of aldrin, (4) approximately 30% unaccountable loss of dieldrin. It is unlikely that the losses were due to volatilization, leaching, or mechanical illuviation. Some sorption phenomenon may be involved, and perhaps some microbial transformation may have occurred after nearly 2 years.

DISCUSSION.—Aldrin and dieldrin recoveries do not appear to correlate with buffer capacity, ammonium-binding power, cation exchange capacity, mechanical analysis, moisture tension, pH or chemical analyses of the soils. No single soil property appeared responsible for reported variations in effectiveness of the two insecticides. Properties found sufficiently different in the two soils to indicate that they may be concerned with this problem are their cation exchange capacities and their calcium and magnesium contents. However, in view of the water-insolubility and non-polar nature of aldrin and dieldrin, no explanation on either of these bases can presently be offered.

Table 7.—Aldrin and dieldrin recovered from Chehalis silt loam soil after 623 days in field cells.[a]

Bunker	Found (p.p.m.) Aldrin[b] Added	Found[c]	Dieldrin[b] Added	Found[c]	Unaccounted for (p.p.m.)[c]
Aldrin	5.4	1.15±0.15	0.0	1.34±0.11	2.51±0.27
Dieldrin	0.0	0.35±0.16	5.0	3.04±0.24	1.62±0.24
Control	0.0	0.0	0.0	0.0	0.0

[a] Phenylazide method of analyses, single extraction with Skellysolve B plus acetone for each toxicant. Sensitivity of method: 0.2 and 0.4 parts per million for aldrin and dieldrin, respectively.
[b] Added September 29, 1954; found June 14, 1956.
[c] Mean ± standard error.

Limitations of the standard extraction procedure for aldrin with Skellysolve B indicate that the soil has some binding property for the toxicant. Unfortunately this was not detected until late in our studies and earlier analyses must be regarded as too low. Nevertheless the data suggest apparent losses and possible transformations. The benzene plus isopropanol extractant used in bioassay and total chloride methods appears to be a better solvent than Skellysolve B for aldrin because the toxicant concentrations found at 0 time agreed within experimental error with the amount of toxicant added (Kiigemagi *et al.* 1958). The total chloride and bioassay methods did not indicate any immediate changes in the toxicant. Such changes were shown with the dieldrin solvent (Skellysolve B plus 25% acetone) within 1 day after insecticides were mixed with the soil. It would appear that none of the methods used in these studies give a complete picture of what happens to the insecticides after their contact with the soil.

CONCLUSIONS.—Although the principal objective of this investigation, to account for differences in insecticidal performance in different soil types, has not been realized, information of interest has been obtained. The study shows that there is a definite need for a more realistic evaluation of what happens to insecticides after they are mixed with the soil.

After approximately 21 months in the field, nearly one-half of the 5.4 p.p.m. aldrin added to the soil was lost, although a significant amount was recovered as dieldrin. Dieldrin loss from dieldrin-treated plots was less than aldrin loss from aldrin-treated plots, and no significant transformation to aldrin was detected.

In sterile soils, traces of aldrin were found with dieldrin treatments; this raises the question of non-microbial transformation.

The phenylazide methods used for aldrin and dieldrin in soil extracts were sensitive to 1 and 2 micrograms, respectively. With 20-gram soil samples this was equivalent to 0.2 p.p.m. for aldrin and 0.4 p.p.m. for dieldrin. This corresponds to the sensitivity and reproducibility for the methods when applied to animal tissues (Bann *et al.* 1956).

The method for extraction of aldrin from soil needs additional investigation. Successive extractions with aldrin solvent gave higher recovery. The dieldrin solvent, Skellysolve B plus 25% acetone, rather than Skellysolve B alone, gave greater recovery of aldrin from soil with a single extraction.

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Development of an Alfalfa Having Resistance to the Meadow Spittlebug[1]

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ABSTRACT

Purdue Synthetic F. alfalfa, assigned the number A600 by the Alfalfa Improvement Conference has been synthesized for evaluating resistance to certain insects as well as for desirable agronomic qualities. Numerous field trials of 2 acres in size show that resistance to the meadow spittlebug found in three of its four parent clones is transmitted to the progeny. A600 is not immune, but has a high amount of resistance not found in any commercially grown alfalfa. This resistance appears to be of three types: antibiosis, tolerance, and possibly unattractiveness or non-preference. It appears to inherit antibiosis from one of its parent clones, C40, which has consistently supported much lower populations than other clones in survival and field population studies. Tolerance is probably the most important resistance factor in A600, since damage from infestation is generally slight. Unattractiveness, or non-preference, is also probably a factor. There has been some evidence of this in selected alfalfa, but it is more difficult to ascertain.

This paper represents a continuation of the work reported by the authors (1952, 1953) in which they showed differences among alfalfa selections in resistance to the meadow spittlebug, *Philaenus leucopthalmus* (L.). They studied spittlebug populations, their damage to the plants and the effect on yield and found significant correlations between populations and damage and between damage and yield. From the data it was concluded that alfalfa could be selected for degree of resistance or unattractiveness illustrated by some selections which supported low populations, in contrast to other alfalfa which was host to very high populations. It was also concluded that alfalfa could be selected for high tolerance to spittlebugs. Some selections of alfalfa which had large populations of spittlebugs received little damage from them.

Since the publication of these papers, an intensified study of the selections appearing to have the most resistance has been made. Polycross seed from various clones was planted in special uniform nurseries arranged in a Latin square for study. This was done because of the tremendous variability that occurs under natural infestation in the field which makes population studies difficult. Survival studies were made, the results of which suggest antibiosis as a factor for resistance in some clones.

To determine if resistance found in small plots would

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carry over into large plantings, an alfalfa synthetic was made consisting of four clones, three of which were selected for insect resistance, including resistance to the potato leafhopper, *Empoasca fabae* (Harris). All three clones had some resistance to both insects. However, C40 had the highest degree of resistance to the spittlebug while C51 and C53 were the most resistant to the potato leafhopper. A fourth clone having moderate resistance to leafhoppers and resistance to *Pseudopeziza* leaf spot was included in the synthetic. This synthetic, labeled Purdue Synthetic F, has now been assigned the number, A600 by the Alfalfa Improvement Conference. It was multiplied in 1955 in California and returned to Indiana for seeding in 2-acre plots throughout the State in 1956.

This paper will be confined to reporting the entomological studies involved in determining resistance to the meadow spittlebug found in the parent clones and their progeny, the synthetic strain.

METHODS.—There are several factors which work against the rapid selection of alfalfa for resistance to the meadow spittlebug. First, since the insect has only one generation a year, work has to be intensified into a period of only 4 to 6 weeks each spring. Secondly, the insect does not seem to adapt well to greenhouse studies, and thirdly, it has not been possible to evaluate alfalfa for resistance to this insect in the early stages of the breeder's program. The latter has been due to low populations resulting from the unnatural environment which exists in space planted and row cultivated nurseries.

To obtain an adequate population of meadow spittlebugs the alfalfa should be planted with a small grain companion crop since infestation is dependent upon the presence of straw and stubble in which the insects oviposit. Also, in the cultivated nursery the spittlebugs are more exposed to desiccation by the wind than in a bulk seeding and populations are reduced. Consequently, selection for spittlebug resistance has been confined largely to broadcast nurseries of advanced progenies. Studies are being continued at Purdue to find more rapid methods for spittlebug screening in the earlier stages of the breeding program.

EVALUATION OF SPITTLEBUG RESISTANCE IN PARENT CLONES.—Initial spittlebug evaluations were made in all cases from plots of alfalfa grown in broadcast nurseries from open pollinated seed produced on selected clones. Plots consisted of five rows 22 feet long seeded 7 inches apart. Between plots there was an interval of 21 inches. For concentrated study, selected material, along with check varieties, was seeded in broadcast nurseries made up of eight entries arranged in a Latin square. Individual plots were only 4×5 feet because of a low supply of seed.

The following technique has generally been used for sampling spittlebug populations in uniform nursery plots. Three-square-foot samples were taken at the same location in each plot, one approximately 2 feet from each end and one in the center of each plot. These were averaged to give the population of spittlebugs per square foot for each plot. In this way data were obtained on the number of spittlebug masses, and the number of spittlebugs per mass as well as the number of spittlebugs in a unit area.

Spittlebug damage which is manifested by a shortening of the plant internodes and a bunchy rosetting type of growth to the stem was evaluated by using a scale of 1 to 9 to rate the plots. A rating of 1 to 3 indicated slight or no injury, 4 to 6 moderate injury, while the 7 to 9 classification indicated severe stunting and rosetting of the plants. A comparison of damage to the three parental clones having resistance with three susceptible varieties and the susceptible check clone, C42, is shown for a period of 5 consecutive years in table 1. For simplification, numerous other entries studied in these tests are not shown. In all tests where the above mentioned selections were entered, the analysis of variance has indicated differences that were significant at the 1% level.

ANTIBIOSIS STUDIES.—Tests were conducted to determine if antibiosis was a factor conditioning resistance in alfalfa to the meadow spittlebug. Cuttings were made from selected clones and potted 6 months in advance of study so that the plants were well established at the time of infestation. Each clone was replicated 10 times and each pot infested with 20 first-instar nymphs collected in the field. Tests were conducted in an open screened insectary.

Observations were made every hour during the first day following innoculation and then once each day for a period of 3 weeks. In all three tests conducted in 2 different years there was a high mortality of nymphs with an average of 52% surviving. This may have been due to natural mortality or possibly due to the change in environment effected by the transfer from the field to individual plants where the nymphs were more exposed, particularly to the drying effects of the air. However, in all tests, less than half as many nymphs survived on C40 as on the susceptible check, C42.

The following observations were made during the first few hours following innoculation. Within the first hour the nymphs settled down on the susceptible check, C42, and began to secrete spittle. Within a few hours this spittle became very abundant. In the case of C40, very little spittle was secreted the first few hours. The nymphs that did become established secreted spittle throughout their development, but it was observed that the spittle was not secreted so copiously as on the C42 plants. It was also observed that on C40 plants the nymphs tended

Table 1.—Comparison of meadow spittlebug damage to an open-pollinated seeding of three parent clones of A600 with three common varieties and an open-pollinated seeding of a susceptible check clone.[a]

SELECTION	1951	1952	1953	1954	1955	MEAN
Ranger	8.5	7.7	7.6	7.3	7.5	7.7
Buffalo	8.2	7.5	6.6	7.9	6.9	7.4
Atlantic	8.5	8.5	6.2	—	—	7.7
C40 (parent clone)	2.0	3.6	2.3	3.0	1.5	2.5
C51 (parent clone)	3.0	5.1	2.9	3.6	4.0	3.9
C53 (parent clone)	5.2	4.7	4.2	3.9	3.6	4.3
C42 (susceptible check)	9.0	8.4	6.0	6.9	6.0	7.3
L.S.D. at 5% level	2.1	2.8	1.9	1.5	1.3	
at 1% level	2.7	3.7	2.5	1.9	1.7	

[a] Damage based on scale rating: 1 to 3 slight, 4 to 6 moderate; 7 to 9 severe damage.

Table 2.—Comparison of meadow spittlebug survival on innoculated clones with field populations on open-pollinated progeny of the same clones.[a]

Clone	Survival Study			Field Study				
	Test 1	Test 2	Test 3	1951	1952	1954	1955	Mean
C40 (parent clone)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
C51 (parent clone)	3.8	2.8	4.5	3.5	4.0	2.5	6.5	3.9
C35 (parent clone)	6.8	2.8	3.5	6.0	3.0	6.5	9.0	5.4
C42 (susceptible check)	6.8	9.0	9.0	9.0	9.0	9.0	8.0	8.5

[a] Data converted to scale rating 1 to 9, (1 to 3 low survival or low field population, 4 to 6 average, 7 to 9 high survival or high field population.

to wander about considerably. Several were observed to leave the plant and walk about on the soil beneath.

Survival data from these studies are shown in table 2 along with field population data obtained from plots of polycross progeny of these same clones. All data have been converted proportionately to a 1 to 9 scale, a rating of *1* indicating a low population or low survival and *9* a high population or high survival. Analysis of original field population data showed differences that were significant at the 1% level. In the survival study, differences were not significant due to considerable variation on some of the plants. However, as is shown in table 2, the data follow the same pattern as in the field studies.

EVALUATION OF SPITTLEBUG RESISTANCE IN A600.—The first seed of A600 was planted in a uniform nursery at the Southern Indiana Forage Farm in 1955, the same year that seed was sent to California to be multiplied for large plot evaluation. Data were obtained from these plots in the spring of 1956 and are shown in table 3. The highest yield was obtained from A600, but it was not significantly greater than most of the other varieties. Although there were practically no spittlebugs on A600 which averaged 18 per square foot, the range of 35 to 60 in plots of the other varieties represented a border-line economic infestation. Weaver *et al.* (1952), Weaver (1954), and Everly (1953) have shown that infestations averaging less than one nymph per stem are border-line economic infestations. Based upon approximately 50 stems per square foot, infestations in this experiment would be in this range.

Table 3.—Comparison of meadow spittlebug populations and damage rating on A600 alfalfa with nine varieties. Southern Indiana Forage Farm, May 19, 1956.

Variety	Population Rating[a]	Damage Rating[b]
Atlantic	7.8	6.1
DuPuits	8.3	4.8
Grimm	6.1	6.5
Buffalo	6.2	7.7
Vernal	8.1	5.5
Williamsburg	7.0	6.8
Ranger	6.2	6.3
Kansas Common	6.9	7.1
Narragansett	8.0	4.8
A600	1.3	1.5
L.S.D. at 5% level	2.4	2.4
at 1% level	3.3	3.2

[a] Population rating based on converted data (rating of 1 indicates a population of 15 spittlebugs per square foot, 9 indicates 50 or more spittlebugs per square foot).

[b] Damage rating scaled 1 to 9: 1 to 3 slight or no damage, 4 to 6 moderate, 7 to 9 severe.

Table 4.—A comparison of infestation by the meadow spittlebug in 2-acre plots of A600 alfalfa with check varieties.[a]

Location	Per Cent of Stems Infested		Nymphs per Infested Stem		Field Population (Nymphs per Stem)	
	A600	Check	A600	Check	A600	Check
Rising Sun	88	92 (B)	3.9	6.3	3.4	5.8
Charlestown	76	96 (B)	2.6	4.8	2.0	4.6
Wheeling	56	72 (R)	2.4	4.5	1.2	3.2
Kewanna	40	72 (R)	2.2	4.3	0.9	3.0
Cuzco	40	68 (R)	1.6	2.5	0.6	1.7
Gosport	36	80 (A)	1.6	2.4	0.6	1.9
Corydon	32	60 (B)	1.5	2.0	0.5	1.2
Buck Creek	30	70 (R)	2.0	3.1	0.6	2.2
		50 (V)		4.1		2.1
Dayton	24	56 (R)	1.0	1.9	0.2	1.0
Shipshewana	16	64 (R)	1.6	1.8	0.3	1.1
Columbus	16	56 (R)	1.0	1.9	0.2	1.1
		36 (B)		1.2		0.5
Mean	41	67	2.0	3.1	1.0	2.3

[a] Check varieties are abbreviated within parentheses as follows: (A) Atlantic (B) Buffalo, (R) Ranger, and (V) Vernal.

In the spring of 1956, A600 was seeded in 2-acre plots by cooperating farmers at 22 locations distributed throughout the State of Indiana so that all general soil types and conditions would be represented. Check varieties were also included. In southern Indiana the check variety was usually Buffalo or Atlantic, while in northern Indiana Ranger was seeded as the check variety. In one case, Vernal was used along with Ranger as a check.

Table 4 presents the data obtained to compare the infestation of A600 with the check varieties at various localities. The stem infestation of A600 averaged 26% less than the check varieties while the population of nymphs was two to four times as great on the check varieties. With the exception of three fields: Rising Sun, Charleston, and Wheeling, spittlebug populations on A600 were not of economic significance. At Rising Sun as the data show, an unusually severe infestation occurred in which all neighboring fields were literally "saturated" with

Table 5.—Comparison of damage by the meadow spittlebug to A600 alfalfa with check varieties.[a]

Location	Per Cent of Stems With							
	No Damage		Slight Damage		Moderate Damage		Severe Damage	
	A600	Check	A600	Check	A600	Check	A600	Check
Rising Sun	50	0	0	10	15	0	35	90 (B)
Charlestown	65	0	25	10	5	20	5	70 (B)
Wheeling	65	15	20	10	15	10	0	65 (R)
Kewanna	72	0	12	24	0	28	16	48 (R)
Gosport	60	0	35	5	5	45	0	50 (A)
Corydon	55	5	25	15	10	55	10	25 (B)
Buck Creek	75	15	5	10	5	20	15	55 (R)
		25		20		20		30 (V)
Arlington	55	5	15	15	5	25	25	55 (R)
Shipshewana	92	52	4	20	0	16	4	12 (R)
Columbus	85	20	15	55	0	20	0	5 (R)
		45		15		25		15 (B)
Mean	67	15	16	17	6	24	11	44

[a] Check varieties are abbreviated within parentheses as follows: (A) Atlantic, (B) Buffalo, (R) Ranger, and (V) Vernal.

spittlebugs. However, even under these unusual conditions, populations of spittlebugs, though very high on A600, were much lower than on Buffalo.

Table 5 presents data which compares damage at each of these localities and shows the high tolerance in A600. An average of 67% of the stems of A600 showed no visible symptoms of damage while only 15% of the stems of the check varieties were undamaged. Even at Rising Sun where 88% of A600 stems were infested and the initial field population of spittlebugs averaged 3.4 per stem, 50% of the stems at time of harvest showed no evidence of damage.

Comparative yields have not been shown. Numerous factors influence yields and an evaluation of yields should include all of the agronomic factors. However, in general, A600 has yielded better than Ranger or Buffalo and equal to Vernal except under conditions of spittlebug infestation. In the latter case it has yielded higher than Vernal under Indiana conditions.

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Persistence of Aldrin and Heptachlor Residues on Alfalfa[1]

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ABSTRACT

Alfalfa was treated in 1956 with heptachlor and aldrin at the rates of ¼ lb./acre and ⅛ lb./acre, in order to study the disappearance of those insecticides under Wisconsin conditions. Samples were collected immediately after treatment and 1, 3, 7, 14, and 21 days thereafter and were analyzed by specific chemical methods and by a direct feeding bioassay, using *Drosophila melanogaster* Meig. as a test insect. Seven days after treatment, both heptachlor and aldrin were recovered at a rate of 0.1 p.p.m., as tested by chemical analysis. However, when bioassays were conducted, the recoveries were slightly higher. In the case of aldrin, 7 days after treatment, 3.5 to 7.6 times more toxicant was recovered by bioassay as compared with results obtained by a specific chemical analysis. No heptachlor was found 2 weeks after treatment as tested by chemical and biological methods. No aldrin was found 3 weeks after treatment as tested by both methods.

Control of injurious insects with insecticides is frequently required on forage crops and the amount of residues remaining on the plants within a given time becomes of primary importance (Decker *et al.* 1950). Federal law prohibits contamination of forage in excess of allowable tolerances.

Since aldrin and heptachlor are both suitable for the control of grasshoppers and mirids on alfalfa (Medler 1955), knowledge regarding the loss of residues of those insecticides following application in relation to dosage and crop growth is needed. Climatic differences in various areas may influence the persistence of those insecticides on forage. The present study was conducted under Wisconsin conditions to provide comparative residue data, where aldrin and heptachlor were used on alfalfa.

PROCEDURE.—At the end of July 1956, when the second growth of alfalfa reached a height of approximately 10 inches, five plots, each 75×120 feet and separated by 50-foot buffer strips were staked out.

On July 31, the plots were sprayed with emulsions of aldrin or heptachlor at the rate of 10 gallons per acre containing ¼ or ⅛ pound insecticide. A 5-h.p. garden tractor was used equipped with a compressed air type of sprayer adjusted to a pressure of 40 p.s.i. The boom width spray pattern of 15 feet was maintained precisely without overlapping by means of temporary guide stakes placed at 15-foot intervals on each side of a plot. The spray delivery of the outside nozzle was measured during the first and last lap of the eight laps needed for plot coverage. The nozzle delivery was used to calculate the actual dosage rates of the insecticides in each plot. The actual applied rates proved to be somewhat higher than the 2- and 4-oz. dosages intended. Aldrin was actually applied at 2.4 oz. and 5.0 oz. per acre, and heptachlor at 2.6 oz. and 4.6 oz. per acre.

Sampling.—Poos *et al.* (1950) showed that alfalfa samples taken on a representative area basis were more dependable than those taken on a representative plant or stem basis. Our alfalfa was harvested with a forage chopper which progressed crosswise of the sprayer path. The harvest included all the plant parts more than 1 to 2 inches above the soil. As the forage chopper moved across a plot, the alfalfa blown out of a spout was sampled at random and placed in pliofilm bags. The weight of one bag with a sample amounted to 2500 to 4000 grams. In order to prevent contamination the machine was cleaned of alfalfa between each plot, and the untreated check plot

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was cut first at each sampling date. Samples were taken immediately after treatment and at 1, 3, 7, 14 and 21 days following application of the insecticide. Within one-half hour after sampling, the alfalfa was stored in a freezer, pending analysis.

WEATHER CONDITIONS.—Rainfall and mean temperatures recorded during the sampling period (July 31 to August 21) are presented in table 1.

CHEMICAL ANALYTICAL METHODS.—*Aldrin.*—Two hundred and fifty grams of alfalfa were finely ground in a food chopper and then mixed with 250 grams of anhydrous sodium sulfate. This mixture was then placed in a freezer for 24 hours to assure more complete removal of water into the sulfate complex. For extraction an equivalent of 200 grams alfalfa was placed into 2-quart Mason jars, a mixture of redistilled hexane and isopropanol (2:1) was added (4 ml. solvent per gram of material) and a head to end tumbling was then applied for a period of 1 hour.

After tumbling the supernatant liquid was decanted through filter paper and the recovery volume was recorded, to be used as a factor for calculating the results. The isopropanol was removed by washings with water and then a saturated solution of sodium chloride. The alcohol free phase was dried over anhydrous sodium sulfate. Cleanup was done by chromatography with an Altasol-celite mixture. Analysis was done in duplicate by the phenylazide method (Danish *et al.* 1950, O'Donnell *et al.* 1954). The analytical results were corrected for apparent aldrin and expressed in parts per million, based on the fresh weight of the alfalfa samples.

Heptachlor.—Two hundred grams of alfalfa were finely ground in a food chopper and then mixed with 400 grams of anhydrous sodium sulfate. This mixture was placed in a freezer for 24 hours. An equivalent of 50 grams of alfalfa was extracted in a soxhlet extractor, using a mixture of colorimetric grade pentane and acetone (4:1) as solvent. Samples were extracted for 5 hours.

After extraction, the acetone was removed by washings with water and then with a saturated solution of sodium chloride. Cleanup was done by chromatography, using Florex as an adsorbent.

Analysis was done according to the Polen-Silverman method (Polen & Silverman 1952, Ordas *et al.* 1956). Two extractions and analyses were made and the results were corrected for apparent heptachlor and expressed in parts per million, based on the fresh weight of alfalfa. Known amounts of heptachlor added to untreated alfalfa resulted in a recovery of 91 to 94%.

BIOASSAY.—All samples under investigation were bioassayed by a direct feeding method using *Drosophila melanogaster* Meig. as a test insect. The information thus obtained gave an estimate of the amount of toxicant present in a particular sample and possibly indicated the presence of a toxic metabolite or breakdown product of the insecticide, not measured by a specific chemical analysis. Direct feeding methods are being used by various workers (Sun *et al.* 1954, Dewey 1955, Wylie 1956,

Table 1.—Rainfall and mean temperatures at the University Hill Farm, Madison, Wisconsin during the period July 31–August 21, 1956.

DATE	SAMPLING DATES	RAINFALL (INCHES)	MEAN TEMPERATURE ° F.
July 31	+		62
Aug. 1	+		64
2		0.16	65
3	+		65
4			70
5		0.16	79
6			71
7	+		69
8			72
9			71
10		0.44	70
11			69
12		1.18	68
13			71
14	+		69
15			73
16		0.05	73
17		0.09	70
18		0.01	66
19			56
20			55
21	+		58
Total		2.09	

Table 2.—Recoveries of heptachlor and aldrin from alfalfa at various intervals following application.

SAMPLING[a]	CHEMICAL ANALYSIS		BIOASSAY		RATIO BIOASSAY: CHEMICAL	CHEMICAL ANALYSIS		BIOASSAY		RATIO BIOASSAY: CHEMICAL
	p.p.m.	Per Cent	p.p.m.	Per Cent		p.p.m.	Per Cent	p.p.m.	Per Cent	
	Heptachlor 4.6 oz./acre					*Aldrin 5 oz./acre*				
Initial	13.30	100	19.4	100	1.46	6.02	100	9.18	100	1.52
1	4.67	35.2	7.78	40.0	1.67	3.98	66.0	4.06	44.2	1.02
3	0.73	5.5	1.61	8.4	2.25	0.47	7.8	1.19	13.1	2.52
7	*0.10*	0.75	*0.16*	0.84	1.60	*0.06*	1.0	*0.46*	5.0	7.56
14	0.00	0.00	0.00	0.00		0.00	0.0	0.13	1.4	
21	0.00	0.00	0.00	0.00		0.00	0.0	0.00	0.00	
	Heptachlor 2.6 oz./acre					*Aldrin 2.4 oz./acre*				
Initial	3.76	100	15.4	100	4.10	4.13	100	4.65	100	1.13
1	1.05	27.9	5.44	35.4	5.20	1.82	44.0	2.44	52.3	1.35
3	0.30	8.0	1.36	8.8	4.52	0.37	9.0	0.81	17.4	2.18
7	*<0.10*	0.7	*<0.10*	0.54	1.00	*0.10*	2.4	*0.35*	7.5	3.50
14	0.00	0.0	0.00	0.00		<0.10	1.2	0.18	3.9	
21	0.00	0.0	0.00	0.00		0.00	0.0	0.00	0.00	

[a] Days after application.

Edwards *et al.* 1957). Since each material under investigation presents different problems, a method was worked out (Lichtenstein & Harper, unpublished) which permitted a direct exposure of the flies to the treated alfalfa. This eliminated an extraction procedure, which is time consuming and influences to some extent the accuracy of the analyses. By this direct feeding method 0.1 p.p.m. of toxicant could be detected.

All results are means of six different analyses and are expressed in p.p.m., based on the fresh weight of the alfalfa.

RESULTS AND DISCUSSION.—Table 2 summarizes the analytical results in p.p.m. and in per cent of the initial analytical findings.

Figure 1 shows the recoveries of heptachlor and aldrin ($\frac{1}{4}$ lb./acre application) in p.p.m. over a period of 14 days.

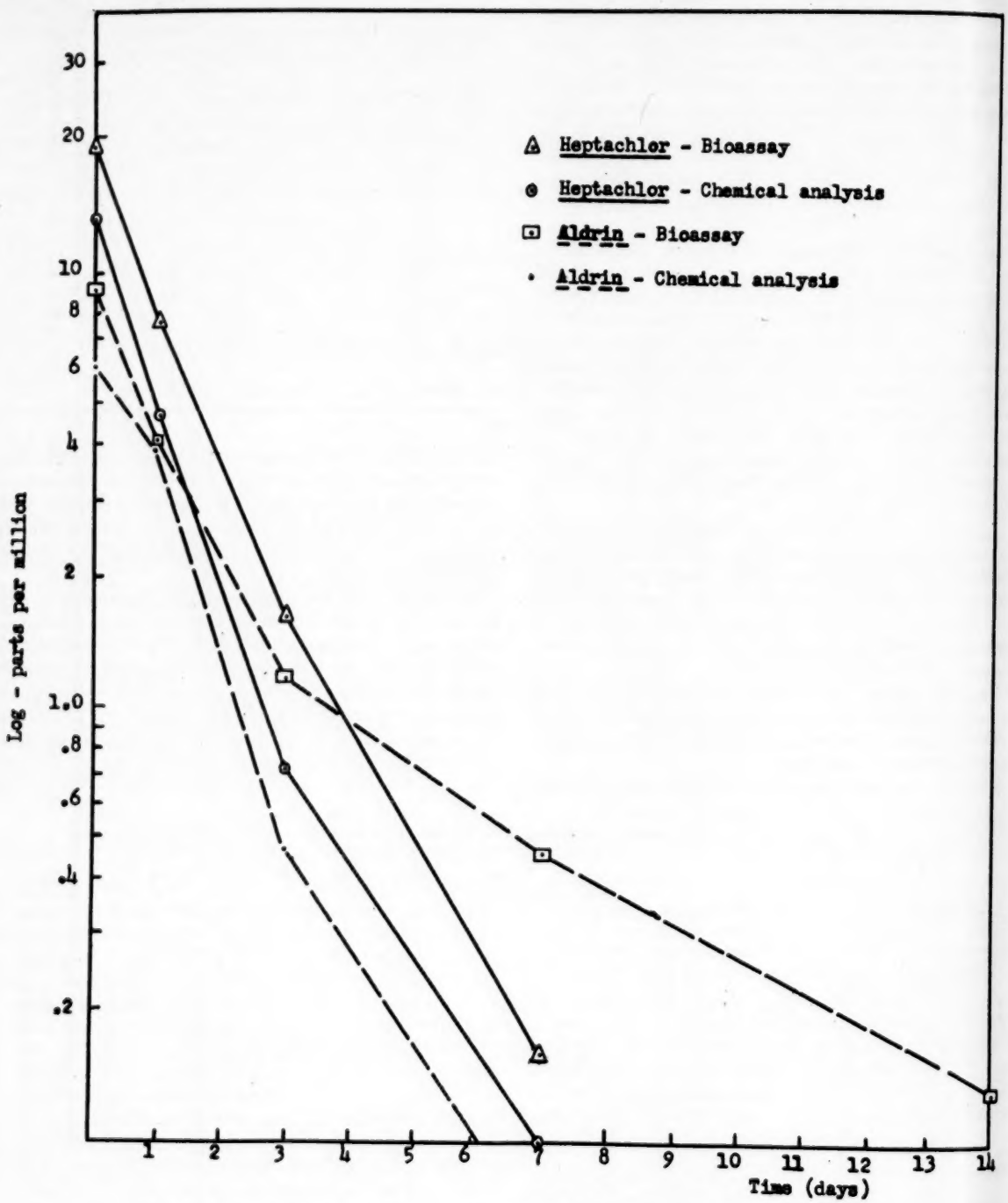

FIG. 1.—Recoveries of heptachlor and aldrin in p.p.m. ($\frac{1}{4}$ lb./acre application to alfalfa).

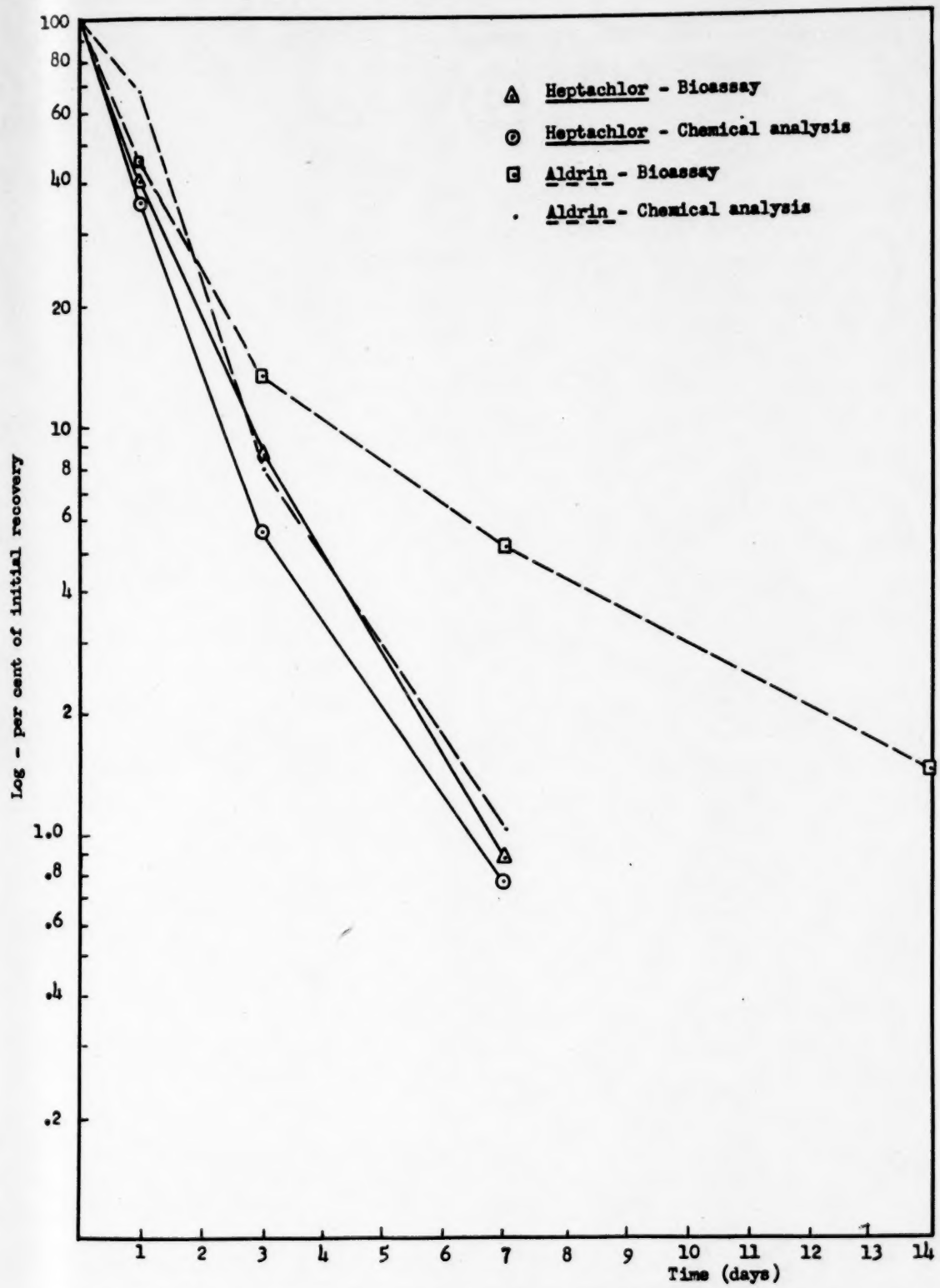

FIG. 2.—Recoveries of heptachlor and aldrin (¼ lb./acre application to alfalfa) in per cent of initial residue data.

Figure 2 shows the recoveries of heptachlor and aldrin (¼ lb./acre application) over a period of 14 days in per cent of the analytical results obtained from the sample collected immediately after treatment.

Heptachlor and aldrin, applied at the rate of ¼ lb./acre, were recovered at a rate of 0.1 p.p.m., and <0.1 p.p.m., respectively, 7 days after treatment, as tested by specific chemical analyses. At a rate of ⅛ lb./acre, less than 0.10 p.p.m. of heptachlor and 0.10 p.p.m. of aldrin were recovered after 7 days as tested by specific chemical analyses. However, when the alfalfa was bioassayed, the amounts of toxicants found were slightly higher as compared with the chemical analyses (table 2).

Two weeks after treatment, no heptachlor was found on alfalfa by either method. However, aldrin residues—as tested by bioassay, still amounted to 0.13 to 0.18 p.p.m. after that period. Twenty-one days after treatment no residues were found whatsoever.

Since the ratio of the difference in recoveries of aldrin, as tested by bioassay and by a chemical method, became larger in each subsequent sampling (table 2, figs. 1 and 2) it seemed likely that part of the aldrin was converted into another toxicant, not revealed by the chemical analysis.

When heptachlor or heptachlorepoxide were added to macerated alfalfa, LD-50 values for *Drosophila* were found to be 1.29 p.p.m. for heptachlor and 2.59 p.p.m. for heptachlorepoxide.

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Biology and Control of the Leaf Curl Plum Aphid in Northern California[1]

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ABSTRACT

In 1953, the leaf curl plum aphid, *Aphis helihcrysi* Kalt., caused considerable damage to plums in the foothill areas of California. Biology and control studies were undertaken in 1954 and continued through 1957.

The aphid, although having a very extensive host range, is closely associated with plum in California. It overwinters as an egg located between or behind the buds. The eggs hatch about the petal fall stage of the plums, and the stem mothers give rise to small green apterous forms which tightly curl the leaves. The aphids continue to reproduce within the curled leaves, infesting new growth until early June. At that time, alates are produced which leave the plums for an unknown alternate host. In late fall, alates return to the plums and give rise to small brown oviparae which mate and lay the overwintering eggs.

Oil-dinitro treatments were effective in controlling the overwintering eggs, but injured some plum varieties. Oil-phosphate sprays at the delayed dormant stage also controlled the eggs and did not injure the trees. With the exception of Thimet, no phosphate tested gave control of the aphids once they were within the curled leaves.

The leaf curl plum aphid, *Aphis helichrysi* Kalt., has probably been present for many years in California, but it was not until 1952 that it was observed in damaging numbers on plum. At that time, the infestation was limited to Placer County, which is located in the Sierra foothill district of California. Since that time, it has been observed as a pest of both plums and prunes in the upper Sacramento valley as well as the foothill districts.

In other areas of the United States however, the leaf curl plum aphid has been a pest of plum and prune for many years. One of the first reports was by Smith (1921) in Idaho who stated that red clover and aster were heavily infested. The aphid is also present in Colorado, where Mickle & Newton (1951) developed methods of control. Kearns & Morgan (1950) discussed the damage done by this pest in England, and Bennett (1955) worked out a very complete life history study in that country.

The leaf curl plum aphid has a very wide host range,

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and control studies were undertaken by Smith *et al.* (1950) on chrysanthemum, as well as by Jefferson (1951) on seed flower crops, notably *Centaurea cyanus* L.

Even though this aphid has a very wide host range, it seems to be found principally on plums in the foothill districts of California, and its seasonal history is closely associated with this tree fruit.

The damage caused by this pest is quite different from that associated with most aphid species. The aphid infests the leaves, and curls them very tightly. Honeydew is produced, but it hardens rapidly to a granular form, and therefore does not drip to the foliage or fruit. A heavy infestation of the leaf curl plum aphid can curl almost every leaf on a tree, and result in a reduction of fruit size as well as producing irregular growth in the shoots and twigs. This latter characteristic results in a pruning problem during the winter months. It has also been reported by growers that a heavy infestation reduces the number of fruit buds for the following season. Studies on both the biology and control of the leaf curl plum aphid were carried out concurrently during the 1954 season and continued through 1957. The two phases will be discussed separately in this paper, however.

BIOLOGY IN THE FIELD.—The leaf curl plum aphid overwinters on prunes and plums as an egg. The eggs are found between the fruit bud clusters or behind the leaf buds. The majority of the winter eggs are deposited on new growth, and since most of this wood is normally pruned, only a relatively few eggs survive to the following spring. The eggs are the typical shiny black, elliptical eggs characteristic of most aphid species, and cannot be distinguished from those of the mealy plum aphid *Hyalopterous pruni* (Geoffroy) or the waterlily aphid *Rhopalosiphum nymphaeae* (L.) which also overwinter as eggs on plum and prune.

The eggs hatch in late March, which is during the bloom or petal fall stage of plums in the foothill areas. The stem mother is an apterous, viviparous form which varies in color from dark brown to dark purple. The time of egg hatching differs from that reported by other workers. Smith (1921) states that the eggs hatch during the late dormant season in Idaho, while in England, according to Bennett (1955), the eggs hatch in the fall and the stem mother is the overwintering form. Soon after the stem mothers hatch from the eggs, they produce large numbers of yellowish green apterae which immediately begin to curl the leaves. A rapid build-up of populations occurs within the curled leaves, and as new growth is produced the aphids move to the more tender foliage. As a result, the terminal growth is tightly curled, and as the season progresses, the infestation spreads throughout the tree. Very often, the early stages of attack are limited to one or two twigs on the tree, but within a period of 30 days the entire tree can become infested.

Even though the build-up of this aphid is very rapid, no alate forms are produced in the early season, and thus there seems to be little migration from tree to tree. Frequently uninfested trees can be found adjacent to heavily infested trees, and such trees often remain free of aphids for the entire season. There are limited infestations which appear to be the result of spread within the orchards, but these cases may be due to the aphids being carried by birds or possibly wind.

The aphids continue to reproduce apterous forms within the curled leaves until early June, and at this time, alate forms are produced which leave the plums for a summer host. By the end of June, all the aphids have left the plums, and as a result, subsequent foliage development is normal. This produces a striking pattern which is illustrated in figure 1.

Although a large number of host plants are listed for this aphid, the summer host has not been found in California. Numerous plants from within and outside the orchards were examined during two summers, and with the exception of a single aphid taken from wild carrot (*Daucus* sp.) the summer form of the leaf curl plum aphid was not found. In late November and early December, alates of the leaf curl plum aphid were again found on plums. The fall migrants appear on the foliage, where they give rise to small brown sexual forms which mate and lay the overwintering eggs. In many cases, the fall migrants were not present on all of the trees in any one orchard. There seemed to be no preference for individual plum trees over others, however, and in some cases the center trees would harbor most of the fall migrants, while in other cases the aphids would be concentrated along one side or the other of the orchard. More commonly, the aphids would be scattered at random throughout the orchards.

Even though the aphids do not seem to show a preference for one tree over another, there is a definite preference for certain plum varieties. Ace, Duarte, Kelsey,

FIG. 1.—Infested branch, showing normal growth after aphids leave in June.

Tragedy and Beauty are commonly attacked, but Santa Rosa and President, two of the more important plum varieties in California, seem to be free of leaf curl plum aphid attack.

CONTROL STUDIES.—Experimental plots were established for the control of leaf curl plum aphid during the 1954 season. Since at that time the biology of the pest was not completely known, it was decided to try treatments at various timings, from the dormant period through the spring. The plot was set up in an Ace plum orchard which had suffered heavily the previous season. Each treatment consisted of single tree plots with eight replicates, and the materials were applied with conventional ground equipment and hand guns. Treatments were made at the dormant period in January, the delayed dormant period in February, pre-bloom stage in early March, and the petal fall period in late March.

The treatments were first evaluated in early April by counting the number of infested curled leaves on the trees, and subsequent counts were made by counting the number of infested shoots on each tree. The materials, dosages, time of application and leaf counts are summarized in table 1.

The results show that both the dormant and delayed dormant treatments were effective in destroying the overwintering eggs. The pre-bloom phosphate sprays and nicotine sulfate also gave good control. The treatments at the petal fall stage however, did not control the aphids, because by that time the aphids had already established active colonies and had curled the leaves. Not only do the curled leaves protect the aphids, but since this aphid can reproduce so rapidly, nearly 100% reduction is needed in order to prevent build-up by early summer.

A few of the plots which were free of aphids in the early season developed a few infested shoots by mid-May. These instances, however, involved only a few shoots, and were probably due to accidental spread of the aphids from heavily infested trees.

During the 1955 season, observations were made on several large scale plots established by growers, and in all cases, the use of a dormant or delayed dormant spray resulted in good aphid control. There were several reports however, that the use of oil-dinitro during the winter caused injury to some plum varieties, principally Duarte and Kelsey.

In order to test some materials which might be less injurious to the trees, another series of plots was established during the 1956 season. Unfortunately, the orchard selected for the experimental plot had such a low aphid population the following season that it was impossible to evaluate the treatments.

During the early summer of 1956 an infested orchard was located, and it was decided to try some of the new phosphate chemicals as foliage sprays. The 1954 data had indicated that parathion, malathion, and demeton were not effective in controlling aphids within the curled leaves, therefore three new materials, Thimet, Trithion, and Diazinon were selected for trial. Each plot consisted of four trees with three replicates, and the materials were applied with conventional ground equipment and hand guns.

The treatments were evaluated 2 weeks after application by selecting 25 curled leaves from each treatment, and counting the live aphids present. Materials used, dosages, and the aphid counts are summarized in table 2.

Table 2.—Foliage treatments for control of leaf curl plum aphid. Auburn, Calif. 1956.[a]

MATERIAL	PINTS PER 100 GALLONS	AVERAGE NO. LIVE APHIDS PER LEAF
Thimet	2 of 48% emulsion	0.0
Diazinon	2 of 25% emulsion	7.2
Trithion	1 of 50% emulsion	120.0
Untreated	—	180.8

[a] Treatments applied on May 16, sampled on June 1.

Thimet was the only material which gave complete control, and new growth developed normally on these plots. Trithion did not reduce the populations to any appreciable degree, and even though Diazinon did reduce the aphids to a fairly low level, the new growth was soon infested, and within a month after application there was little difference between this plot and the check.

In 1957, another plot was established to evaluate several oil-phosphate combinations as delayed dormant sprays for aphid egg control. Plots were established in an Ace plum orchard which had indicated a potential population by observations made on the fall migrants during November of 1956. Each treatment consisted of four trees

Table 1.—Control of leaf curl plum aphid. Auburn, Calif. 1954.

MATERIAL	DOSAGE PER 100 GALLONS	DATE APPLIED	NO. OF CURLED LEAVES	NO. OF CURLED LEAVES INFESTED	NO. INFESTED NEW SHOOTS		
			April 12	April 12	April 26	May 18	June 7
Dormant oil+ dinitro phenol	3 gal. 1 lb.	Jan. 13	0	0	0	13	18
Dinitrobutylphenol	1 qt.	Feb. 2	0	0	0	2	7
Parathion	1 lb.	Mar. 2	0	0	0	0	3
Malathion	2 lb.	Mar. 2	0	0	0	3	8
Light medium oil+ nicotine sulfate	1.5 gal. 1 pt.	Mar. 2	6	3	0	0	4
Parathion	1 lb.	Mar. 23	85	13	21	143	268
Malathion	2 lb.	Mar. 23	52	14	29	184	329
Demeton	1 pt.	Mar. 23	60	11	22	225	359
Untreated	—	—	159	141	241	860	1184

with three replications. The phosphate-oil combinations were applied in early February, using conventional ground equipment with hand guns. The plots were evaluated on May 1 and May 29 by counting both the number of trees infested and the number of infested shoots on each tree. The materials used, dosages, time of application, and aphid counts are summarized in table 3.

The results indicate that all of the oil-phosphate combinations were effective in destroying the overwintering eggs. With but one exception, all of the treated trees remained free of curled leaves. The single tree infested in the Trithion-oil plot on May 29 was probably due to reinfestation from a tree in a nearby check plot, since the plot was free of aphids on May 1.

Table 3.—Control of leaf curl plum aphid on Ace plums. Auburn, Calif. 1957.

		Aphid Counts					
		May 1			May 29		
		Number of Trees		Number Infested Shoots	Number of Trees		Number Infested Shoots
Materials[a]	Dosage per Acre	Infested	Clean		Infested	Clean	
48% trithion + dormant oil	3 pints + 6 gallons	0	12	0	1	11	4
10% Phostex-oil miscible	3 gal'ons	0	13	0	0	13	0
5% Nialate[b]-oil miscible	3 gallons	0	12	0	0	12	0
25% parathion + dormant oil	6 lbs. + 6 gallons	0	12	0	0	12	0
Untreated	—	9	8	162	9	8	440

[a] Applied February 7.
[b] Methanedithiol, bis(O,O,diethyl phosphooridthioate (also called Niagara 1240).

These studies suggest that a spray directed against the overwintering eggs will give the most effective control. Oil-dinitro may be used, and in cases where plum varieties are sensitive to this combination, a delayed dormant treatment of oil-phosphate may be substituted. Due to the alternate host complication in the life cycle of this aphid, with a migration to plum trees in the late fall, it will probably be necessary to treat each season in order to hold this pest in check.

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Insecticidal Control of the Chicken Body Louse on Range Turkeys[1]

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Abstract

Malathion, lindane and DDT were field-tested on turkey flocks for the control of the chicken body louse, *Menacanthus stramineus* (Nitz.), following preliminary laboratory screening tests.

Applications were made by a rapid technique which involved the use of a row-crop spray boom placed on the ground with the nozzles directed upward and slightly forward. The turkeys were driven through the spray curtain. An average flock of approximately 2,600 turkeys can be treated in an hour.

Malathion applied at 1% concentration and 60 pounds pressure gave 100% kill of lice within 24 hours. Flocks remained louse-free as long as 3 weeks, or until marketed and processed. A 0.5% concentration of malathion when applied at 40 pounds pressure was only partially effective

Lindane gave 100% kill of *M. stramineus* within 24 hours when applied at a 1% concentration and 60 pounds pressure. By the 14th post-treatment day the population of lice had become re-established.

DDT applied at 3% concentration and 60 pounds pressure reduced the population but did not give complete control.

Control of lice on range turkeys has always been a difficult problem because the birds are housed in open shelters precluding the use of roost fumigants. Individual bird treatments used in the past were expensive, laborious and time consuming.

The laboratory screening tests reported herein were designed to determine the efficacy of several insecticides for the control of the chicken body louse (*Menacanthus stramineus* (Nitz.)), the only louse found on Iowa turkeys to date.

Raun & Nelson (1956) placed a crop sprayer on the ground and directed the spray upwards achieving 100%

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control of the chicken body louse on 4,000 tom turkeys, using malathion as a 1% aqueous emulsion.

Of the insecticides tested in the laboratory screening program, three were applied to large flocks in the field using the technique developed by Raun & Nelson.

SCREENING TESTS.—The insecticides used in the laboratory screening tests were chosen for their ready availability and low toxicity to higher animals. In addition, tests were made with a formulation of the common solvent in the experimental insecticides (xylene) and an emulsifying agent (Triton X-100). These chemicals were mixed in proportions equal to those found in the standard DDT emulsion.

The materials were diluted to a 1% stock solution and reduced to parts per million with distilled water. There were six dilutions, 10,000 (1%), 1,000 (0.1%), 100 (0.01% 50 (0.001%), 10(0.0001%), and 1 (0.00001%) parts per million. One milliliter of each dilution was placed in each of three 9-centimeter petri dishes, containing Whatman No. 2 filter paper, and allowed to dry for 1 hour. Five lice were transferred from an infested turkey to each of the petri dishes and then placed in an incubator at 34 degrees centigrade and a mean relative humidity of 48%. The numbers of the dead lice were recorded at each of eight hourly observations thereafter.

Figures 1 and 2 show the comparative results of each material tested as corrected by Abbott's formula (Abbott, 1925).

FIELD TESTS.—Malathion was field-tested on a flock of 2,650 infested tom turkeys in September, 1956. The boom from a row-crop sprayer was placed on the ground in a chute. The spray pump was of the low pressure rotary type, powered from the tractor power-take-off shaft. The sprayer was adjusted to 60 pounds pressure and the nozzles directed to spray at 90 degrees from the surface of the ground. The birds were penned within a temporary fence of woven wire. Groups of approximately 50 birds were driven through the spray by three men using a rope stretched between them. A total of 60 gallons of 1% malathion aqueous emulsion was used. Preparation for the spraying took about 1 hour and the actual application required 23 minutes.

In October 1956 a flock of 2,800 tom turkeys was treated with a 1% aqueous emulsion of lindane. The same type of facility was employed as in the first field test, except that the nozzles were directed to spray approximately 60 degrees from the horizontal in the same direction in which the birds were to travel. The preliminary preparations required about 30 minutes. Thirty-five gallons of the spray mixture were applied in 48 minutes.

Later in October, a third insecticide, a 3% aqueous emulsion of DDT, was applied to 4,000 infested tom turkeys in the same manner as with the lindane treatment (fig. 3). The preparations required 34 minutes and the actual spraying time was 10 minutes. Because only 40 gallons of the solution were used the flock was immediately retreated. The two treatments together increased the spray time to 22 minutes and the consumption of spray material to 80 gallons. Fifty birds from this flock received only one treatment and were held apart from the rest of the flock.

Another infested flock, located a few days later, consisted of 800 toms. Malathion was used on this flock but in a lower concentration applied at lower pressure. A 0.5% emulsion was applied at 50 pounds pressure. The preparation and spraying time involved 1 hour and 6 gallons of material were used.

FIELD OBSERVATIONS.—The method for counting lice on range turkeys was a modification of techniques employed by Alicata *et al.* (1946) and Moore (1952), the feathers being parted around the vent and the observations made. The count was restricted to an estimated

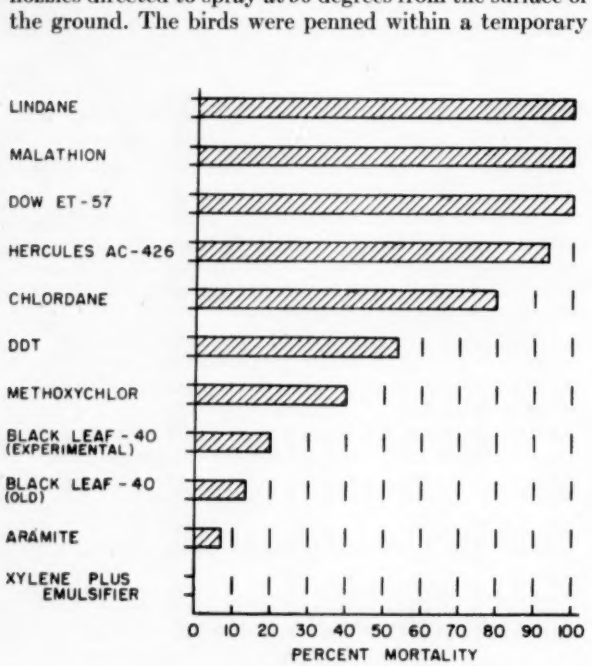

FIG. 1.—Comparative effectiveness of 1,000 p.p.m. (0.1%) concentration of experimental materials against *Menacanthus stramineus* after 3 hours' exposure in laboratory tests.

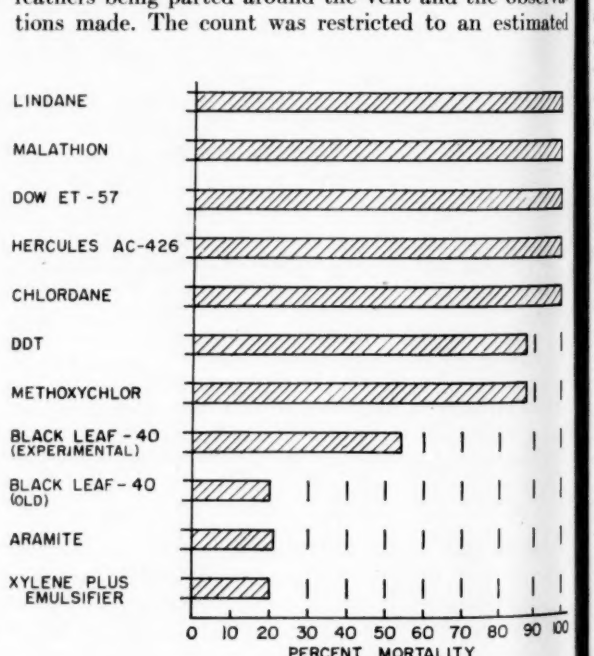

FIG. 2.—Comparative effectiveness of 1,000 p.p.m. (0.1%) concentration of experimental materials against *Menacanthus stramineus* after 5 hours' exposure in laboratory tests.

FIG. 3.—Turkey flock enclosed preparatory to chemical treatment.

area of 4 inches in diameter surrounding the vent. If the visible population was less then 25 the actual number was recorded. Specimens were collected from each flock at the time of first inspection for later indentification in the laboratory.

Before the first malathion field trial, the estimated average infestation per bird was 140 for the five birds inspected. Five birds were removed from the flock to serve as controls. The infestation on these birds averaged 155 lice. On the following day no lice could be found on the 10 birds inspected from the flock, which remained louse-free from the day after treatment to their subsequent date of sale. Louse populations remained contant on the untreated control birds.

The average number of lice for each of 10 birds inspected before the lindane field trial was 41. The day after treatment there were no lice observed. Inspection on the second, third and eighth post-treatment days indicated no infestation. On the 14th post-treatment day 12 small nymphs were observed on three of the 10 birds, inspected. Another inspectation on the 19th day revealed an average of 1.1 lice per bird. On the 21st day the average was 4.9. The larger lice were nearing their last instar on the 26th post-treatment day when the average count reached 12.5. By the 29th post-treatment day, the population was re-established to near the pretreatment normal with an average infestation of 39 lice per bird. At this time it was deemed necessary to retreat the flock. Malathion, 1.0%, applied at 60 pounds pressure, was used with the same equipment previously employed. No lice were found at subsequent inspections.

The average infestation per bird prior to treatment with DDT was 180 in the main flock and 175 in the 50-bird segment. As mentioned earlier the main flock was sprayed twice while the 50-bird segment was sprayed only once. On the following day the average count was less in the 50-bird segment (80) than in the main flock (100) that had had two applictions. This variation is believed due to sampling error. It is interesting to note that the living lice remaining on the birds were first or second instars. No adults could be found. An inspection made 4 days later indicated a sizable population of lice still present. The flock with two applications of DDT averaged 10 lice per bird and the 50-bird segment which had only one application averaged 30 lice.

Retreatment was made the ninth post-treatment day because the average count was 18 lice per bird for both the main flock and the 50-bird segment. The retreatment was with 1% malathion applied at 60 pounds pressure. The day following the malathion application three very small nymphs were found on two of the 10 birds inspected in the main flock and three on three of the 10 birds inspected from the 50-bird segment. Four days later no lice could be found and the birds were sold louse-free.

Before application of the insecticide for the last field trial (malathion, 0.5% at 40 pounds pressure), the louse counts averaged 102 per turkey. On the sixth post-treatment day the average was 63.8. Since only partial control was obtained at this concentration and at low pressure, malathion was used again but at 1% and 60 pounds pressure. On the first and third days after the second treatment, random inspection of the flock revealed no lice and the birds were sold louse-free.

DAMAGE TO BIRDS.—During the course of these investigations the chicken body louse was observed on all parts of the turkey except the lower legs, the feet and the beak. Young nymphs were seen on the caruncles, dewlap and in heavy infestations, even on the snood. The greatest infestation, however, was always within a 4-inch radius of the vent. The major visible damage to the turkey was inflicted in this particular area.

The bird lice are known to eat everything that can be found near the skin and among the feathers of their host (Waterson 1926). *M. stramineus* has been seen to pierce

FIG. 4.—Machine-plucked turkeys: treated (left), untreated (right).

the dermal papilla of a young feather and actually take a blood meal (Wilson 1933). In these experiments the lice were observed with their heads immersed in the fluid drainage of the anus of the tom turkey. Apparently this was an essential or preferred portion of their diet and could be the reason for their concentration in the anal area.

It appears that during feeding this species of louse actually breaks the epidermis. Large scabs of a yellow material were always present when the lice were found. Apparently these scabs are formed from the exudate produced at the breaks in the skin surface.

In addition to skin breakage, feathers are damaged by the egg laying and feeding activities of the lice. The eggs are glued to the base of the individual feathers in large masses. The weight of these eggs causes a continuous pull on the feather which creates an inflammation of the surrounding tissue. Louse feeding in conjunction with the egg laying sometimes causes the feathers to become ingrown. Occasionally an infection accompanies this condition as was evidenced by pustules.

In the past it was not until the birds were marketed on a grade basis that the monetary reduction caused by the infestation was realized. During the mechanical process of removing the feathers from the birds the scabs were also removed. The skin lesions were then revealed (fig. 4). All birds with this type of injury had to be sold at a lower grade, causing a serious reduction in market receipts. Five days after treatment, the skin lesions of treated test birds were sufficiently healed beneath the scabs to permit their sale at top grade. In all cases of treatment, the scabs were gone after 10 to 14 days.

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Resistance of the Citrus Red Mite to Organic Phosphates in California[1]

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ABSTRACT

Under a program in which demeton was used in a California lemon grove to control citrus red mite, *Panonychus citri* (McG.), and parathion was used to control California red scale, *Aonidiella aurantii* (Mask.), the citrus red mite developed resistance to demeton applications. Laboratory evaluations showed that mites resistant to demeton were also resistant in varying degrees to 14 other organic phosphates. It required 15,000 and 30,000 times the concentration of Trithion, and only 8 and 6 times the concentration of Bio 1240 (Bis 5-(diethoxyphosphinothioyl) mercapto methane) in the spray to deposit sufficient residues to effect 50% and 95% mortalities, respectively, in the resistant strain of mites as compared to the nonresistant. In field tests during 1957 those materials known to be effective in controlling nonresistant citrus red mites were ineffective in groves where resistance had developed through the use of both demeton and parathion.

Field observations in 1954 indicated that the citrus red mite, *Panonychus citri* (McG.), had developed resistance to ovex in the Corona citrus district of California. Growers in this area therefore began using demeton for mite control. At the same time experimental treatment programs including successive demeton applications and applications of demeton alternated with those of other acaricides were initiated to ascertain the number of treatments required to develop resistance in the citrus red mite to demeton in a program where parathion was used to control California red scale, *Aonidiella aurantii* (Mask.), and to ascertain mite resistance to other organic phosphate acaricides.

FIELD STUDIES.—*Methods.*—The field studies reported herein were initiated in a lemon grove at Corona, California, in April, 1954, when citrus red mite populations developed a high degree of resistance to ovex. This grove received one application of parathion each year, from 1951

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to 1957, for control of California red scale. The experimental mite-control plots were 1½ acres in size and were 6 rows wide and 25 trees long. Spray applications were made with high-pressure oscillating boom equipment, according to the four schedules noted in table 1. Re-treatment for mite control was scheduled as soon as feasible after the mite populations exceeded 10 mites per 32-leaf sample.

Discussion.—It is evident from the data of table 1 that the citrus red mite rapidly developed resistance to the demeton treatments. Demeton was no longer effective in schedule 1 (demeton only) after the fourth application, or in schedules 2 and 3 (demeton and ovex, alternated) after the second demeton treatment. In schedule 4 (Aramite and demeton, alternated) some lack of effectiveness of demeton was evident, but the mites were less resistant than those treated according to the other schedules.

Most of the groves in the Corona area were treated with demeton during 1954 and 1955, and the spring treatments in 1956 were relatively ineffective. Laboratory evaluations indicated that mites in plots which received an annual parathion treatment but no demeton treatment were resistant to demeton by 1957. This suggests either that the parathion applications for scale control produced mite resistance to demeton or that resistant mites from the surrounding area moved into the untreated plots. The latter seems to be the most likely explanation, since laboratory evaluations of resistance in mites taken from areas where parathion had been used as many years, but no demeton had been used, showed no evidence of resistance to demeton.

Table 1.—Influence of consecutive and alternate field applications of acaricides on development of resistance of citrus red mite to demeton.

Date of Treatment	No. of Weeks of Control Effected by Treatment[a]			
	Schedule and Number			
	1, Demeton Only	2, Demeton and Ovex, Alternately	3, Ovex and Demeton, Alternately	4, Aramite and Demeton, Alternately
Apr. 23, 1954	*20*[b]	*55*	7	20
June 10, 1954	—	—	*42*	—
Sept. 3, 1954	*37*[b]	—	—	*37*
Mar. 28, 1955	—	—	7	—
May 17, 1955	*30*	13	*27*	13
Aug. 11, 1955	—	*54*	—	*54*
Dec. 15, 1955	*31*	—	—	—
Mar. 2, 1956	—	—	13	—
May 31, 1956	—	—	7[b]	—
July 20, 1956	*5*[c]	—	5	—
Aug. 21, 1956	7	7	7	14
Oct. 10, 1956	7	7	7	—
Nov. 28, 1956	X[d]	X[d]	X[d]	*15*

[a] Numbers in italics indicate number of weeks of control resulting from demeton treatments; other numbers indicate results of alternate treatments.
[b] Ten ounces actual demeton used per acre. In all other demeton treatments 20 ounces of demeton were used per acre.
[c] First ineffective treatment or first evidence that mites were resistant to demeton.
[d] Treatments discontinued.

LABORATORY EVALUATIONS.—*Methods.*—The degree of resistance of the citrus red mite to organic phosphate acaricides was evaluated in the laboratory with field-collected resistant and nonresistant mites. Nonresistant mites were obtained from an abandoned orange grove at San Juan Capistrano, which had not been treated the previous 3 years. In this area oil sprays have been relied on for pest control; therefore, phosphate insecticides had not been used. Resistant mites for laboratory studies were obtained from a Jameson Company orange grove at Corona, which had previously been treated five times with a combination spray containing demeton and parathion.

For evaluation of the degree of resistance in the laboratory, lemon fruits were thoroughly sprayed with a range of dosages of each material by means of a DeVilbis-type sprayer. Eight fruits were treated at each concentration, and 25 adult female mites were transferred to each fruit and exposed to the spray residue for a period of 48 hours. Probit log-concentration mortality curves were established for each material. These curves were used to fix dosage levels in grams per liter of spray required for 50% and 95% mortality.

Discussion.—Results of the resistance studies (table 2) indicate that citrus red mites which developed resistance to demeton from a parathion-demeton program were also resistant in varying degrees to the other organic phosphate insecticides tested. The mites were more resistant to Trithion than to either parathion or demeton. It required 15,000 and 30,000 times the concentration of Trithion, and only 8 and 6 times the concentration of Bio 1240 in the spray to deposit sufficient residues to effect 50% and 95% mortalities, respectively, in the resistant strain of mites as compared with the nonresistant mites.

Table 2.—Relative concentrations of some organic phosphates, in grams per liter of spray, required to deposit sufficient toxicant to effect 50% and 95% mortality of resistant (R) and nonresistant (NR) citrus red mites.

Organic Phosphate	Mortality 50%			Mortality 95%		
	Spray Concentration in Grams/Liter		Ratio, R:NR	Spray Concentration in Grams/Liter		Ratio, R:NR
	NR	R		NR	R	
Trithion	0.0002	3.00	15,000	0.001	30.0	30,000
Iso-Systox[a]	.00007	0.60	8,570	.001	2.9	2,900
Methyl demeton	.001	8.0	8,000	.01	40.0	4,000
Parathion	.006	5.30	883	.03	70.0	2,330
EPN	.0005	0.7	1,400	.005	8.0	1,600
Schradan	.005	10.0	2,000	.01	7.0	700
Phosdrin	.01	3.50	350	.04	24.0	600
Diazinon	.002	0.025	12	.006	2.0	333
Demeton	.0045	1.20	266	.027	5.2	192
Pyrazoxon[b]	.001	0.30	300	.05	2.5	50
Chipman R-6199	.0044	0.50	111	.0066	0.6	91
Hercules AC-528	.0015	0.15	100	.190	3.5	18
Bayer 19639	.008	0.2	25	.05	4.0	80
Malathion	.20	1.70	8	.60	5.6	9
Bio 1240[c]	.004	0.032	8	.016	0.1	6

[a] O,O-diethyl S-2-(ethylmercapto)ethyl phosphorothiolate.
[b] O,O-diethyl-O-(3-methyl-5-pyrazolyl) phosphate.
[c] Bis S-(diethoxyphosphinothioyl) mercapto methane.

Latent Toxicity in the Mediterranean Fruit Fly and the Melon Fly[1]

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ABSTRACT

Experiments were conducted to determine whether insecticides other than the chlorinated hydrocarbons caused latent toxicity. The insecticides were applied as soil treatments to larvae of the Mediterranean fruit fly, *Ceratitis capitata* (Wied.), and the melon fly, *Dacus cucurbitae* Coq. The chlorinated polycyclic hydrocarbon insecticides had a high order of latent toxicity. Aldrin, chlordane, dieldrin, endrin, isodrin, Strobane, and lindane exhibited strong properties of latent toxicity to the Mediterranean fruit fly. No latent toxicity to this insect was exhibited by Dilan, malathion, parathion, Pyrenone, or Pyrolan. Isobornyl thiocyanoacetate was practically non-toxic to the larvae. Latent toxicity was shown by aldrin, chlordane, dieldrin, endrin, and isodrin to the melon fly but to a lesser degree than to the Mediterranean fruit fly. Lindane, malathion, and parathion evinced no latent toxicity to the melon fly.

The term "latent toxicity" was proposed by Tamashiro & Sherman (1955) for the phenomenon in which the full lethal effect of an insecticide, although applied to a larva, does not become manifest until after the insect becomes an adult. It was shown that when the larvae of the oriental fruit fly, *Dacus dorsalis* Hendel, were treated with certain chlorinated hydrocarbon insecticides, many of the surviving emerging adult flies exhibited typical symptoms of poisoning and died. On the other hand, when the larvae were treated with parathion none of the emerging adult flies died. The phenomenon of latent toxicity, therefore, appeared to be associated with the chemical structure of the insecticide.

This study was undertaken in an attempt to discover whether insecticides other than the chlorinated hydrocarbons cause latent toxicity and also to determine the degree of susceptibility to this phenomenon of two other tephritid species in Hawaii, the Mediterranean fruit fly, *Ceratitis capitata* (Wied.), and the melon fly, *Dacus cucurbitae* Coq.

MATERIALS AND METHODS.—The compositions of the compounds tested were as follows: aldrin, recrystallized, 99.3%, m.p. 99.0–100.0° C. with sublimation; chlordane, technical; dieldrin, recrystallized, 99.6%, m.p. 164.0–167.0° C.; Dilan, technical; endrin, recrystallized, 96%, sublimes with decomposition at 205.0° C.; isobornyl thiocyanoacetate, technical; isodrin, recrystallized, 97.5%, sublimes at 98° C.; lindane, recrystallized, m.p. 113.0–113.5° C.; malathion, technical, 95%; nicotine alkaloid, 80%; parathion, technical, 96.5%; Pyrenone, a pyrethrum formulation consisting of 75% technical piperonyl butoxide, 7.5% pyrethrins, and 17.5% inert material, sp.g. 1.028; Pyrolan, technical; and Strobane, technical. Stock solutions of these materials dissolved in redistilled acetone were prepared. Dilutions of these stock solutions with acetone were used in all experiments.

Full-grown third-instar larvae of the Mediterranean fruit fly and the melon fly were used in this study.

Two-hundred-gram lots of dried beach sand which had been sifted through 16-mesh screen were placed in pint Mason jars. Five ml. of an acetone solution containing the required concentration of each insecticide was added and vigorously mixed in with a spatula. The acetone was allowed to evaporate by letting the jars stand for about 6 hours, after which 5 ml. of water was added to provide the larvae with sufficient moisture for pupation. Seven days later, the pupae were taken out of the insecticide-treated sand and, after all particles of treated sand were carefully removed from the puparia, were placed for adult emergence in jars containing clean untreated sand. Water and sugar were placed in these jars to serve as food for the emerging adults.

Three days after the start of emergence, the number of dead and moribund adults in the jar was recorded to determine latent toxicity. Carbon dioxide was introduced into the jar, anesthetizing living flies so that they could be readily sifted out of the sand. Their number was determined, added to the number of dead and moribund adults, and used in the calculation of direct mortality. An aid in determining whether an insect was dead or alive was to drop the insect into a beaker of acetone. A dead or moribund insect would float, while the living insect would quickly sink to the bottom of the beaker, usually kicking its legs vigorously.

In ascertaining direct mortality, the flies that had started emergence but were not normal, that is, those that were only partially out of the puparia or those that were completely out but did not have their wings fully expanded, were not counted as emerged adults. Such morphologically abnormal adults were rare among the untreated check population but were common among the flies treated with certain insecticides.

Dosage was calculated in terms of micrograms of insecticide per gram of sand. Twenty larvae were treated at each dosage level and each test was usually replicated at least three times.

The observed mortalities were corrected for natural mortality by Abbott's formula (Abbott 1925). The median-lethal dosages (LD-50) and their fiducial limits at the $P = 0.05$ level were calculated by the method described by Finney (1952).

RESULTS AND DISCUSSION.—*Mediterranean fruit fly.*—In order to convey statistically the idea of latent toxicity in this paper, the direct toxicity of each material was first determined. The median-lethal dosages together with

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their fiducial limits at the P = 0.05 level were calculated. Latent toxicity was determined by calculating the percentage of adults that had died within 3 days of emergence. This calculation cannot be given in terms of a median lethal dosage. However, total toxicity as expressed by total mortality resulting 3 days after emergence can be graphically portrayed and an LD-50 calculated. Thus, the difference between the LD-50's of the direct and the total toxicities will express the latent toxic effect of the insecticide. Table 1 is a compilation of the toxicity data of aldrin to the Mediterranean fruit fly and exemplifies the type of calculations required to derive this information.

Table 2 summarizes the direct and total toxicity of the insecticides to the Mediterranean fruit fly. The toxicity was considered as direct when the insects were killed prior to completion of the pupal stage or before the emergence of morphologically normal adults. The total toxicity is the sum total of the direct toxicity and the latent toxicity.

Significant latent toxicity was shown by aldrin, chlordane, dieldrin, endrin, isodrin, lindane, and Strobane, all of which are chlorinated hydrocarbons. The total toxicities of these insecticides as exemplified by their median-lethal dosages were significantly greater than their direct toxicities. This increase in mortality was due to latent toxicity.

No latent toxicity was shown by Dilan, a mixture of two DDT relatives; malathion and parathion, both phosphorus-containing materials; nicotine; Pyrenone, a mixture of pyrethrins and a synergist; or Pyrolan, a dimethyl carbamate.

Isobornyl thiocyanoacetate was relatively non-toxic to the larvae of this species. The technical product was used undiluted and 5 ml. was added to 200 grams of sand, resulting in an average mortality of only 52%. Latent toxicity was not exhibited by this material.

Sun (1950) describes the toxicity index as a tool for comparing the relative toxicity of insecticides. In comparing the relative potency of the insecticides acting as both direct and latent poisons, parathion was taken as the standard insecticide and given the arbitrary index value of 100. When compared with parathion in this manner, the direct toxicity indices were: isodrin, 663; endrin, 186; lindane, 33; dieldrin, 30; Pyrolan, 27; malathion, 25; Pyrenone, 8; chlordane, 7; aldrin, 6; Strobane, 0.3; Dilan, 0.2; nicotine, 0.1; and isobornyl thiocyanoacetate, less than 2×10^{-6}.

When compared with parathion the effect of latent toxicity in terms of the total toxicity indices were isodrin, 1059; endrin, 314; lindane 43; dieldrin, 69; Pyrolan, 27; malathion, 25; Pyrenone, 8; chlordane, 23; aldrin, 50; Strobane, 0.4; Dilan, 0.2; nicotine, 0.1; and isobornyl thiocyanoacetate, less than 2×10^{-6}.

There was a high correlation between the presence of morphologically abnormal adults and latent toxicity. This can probably be explained on the basis that the effect of the toxicant overtook the adult just prior to emergence. The abnormal adults were also found more at the higher dosage levels which would tend to confirm this hypothesis. Some abnormal adults were found in all treatments but the number was insignificant in treatments with insecticides showing no latent toxicity. It is interesting to note that in none of the untreated groups of flies were there abnormal adults.

The increase in toxicity in this species due to the latent effect was appreciable. The increases ranged from 8.8 times in the case of aldrin to 1.3 times with Strobane and lindane. Not all of the chlorinated hydrocarbon group showed latent toxicity. Dilan, a mixture of nitrochlorophenylbutane and nitrochlorophenylpropane, showed no increase in toxicity due to the latent effect. This chemical is a relative of DDT which showed very little toxicity to the larvae of the oriental fruit fly (Tamashiro & Sherman 1955). In general, it appears from the insecticides tested in this experiment that the chlorinated polycyclic compounds show the most latent activity.

Melon Fly.—Table 3 summarizes the direct and the total toxicity of a number of insecticides to the melon fly. Although aldrin, chlordane, dieldrin, endrin, and isodrin showed evidence of latent toxicity only chlordane caused a highly significant change in total toxicity. No latent toxicity was evinced by lindane, malathion, or parathion. When compared with parathion the direct toxicity indices were: aldrin, 27; chlordane, 6; dieldrin, 13; endrin, 46; isodrin, 53; lindane, 15; and malathion, 11.

Table 1.—The toxicity of aldrin applied as a soil treatment to larvae of *Ceratitis capitata* (Wied.).

Dosage, µG./Gm.	No. Larvae Treated	Abnormal Adults[a]	Normal Adults[b]		Corrected Mean Per Cent Mortality		
			Dead	Alive	Direct[c] Toxicity	Latent[d] Toxicity	Total[e] Toxicity
1.88	40	13	11	1	70	94	98
1.25	40	5	24	2	35	93	95
0.625	40	8	16	10	35	62	75
0.25	60	12	22	16	37	68	73
0.188	60	8	20	24	27	51	60
0.125	60	6	17	31	20	42	48
0.0625	60	3	15	32	22	33	47

[a] Adults that have only partially emerged from puparia and/or adults that have completely emerged but whose wings are collapsed.
[b] Condition of aduits 3 days after emergence.
[c] Toxicity calculated by considering all normal adults as survivors of larval treatment.
[d] Additional mortality in adult flies surviving direct toxicity.
[e] Toxicity calculated by considering only alive normal adults as survivors of larval treatment.

Table 2.—Summary of direct and total toxicity of several insecticides applied as soil treatments to larvae of *Ceratitis capitata* (Wied.).

Insecticide	Direct Toxicity[a]		Total Toxicity[b]
	LD-50 µg./gm.	Fiducial Limits P = .05 µg./gm.	LD-50 µg./gm.
Aldrin	1.43	0.754–2.71	0.163
Chlordane	1.13	0.945–1.36	0.349
Dieldrin	0.275	0.227–0.322	0.118
Dilan	46.8	40.8–53.6	46.8
Endrin	0.0438	0.0390–0.0493	0.0260
Isodrin	0.0123	0.0106–0.0142	0.0077
Lindane	0.246	0.205–0.295	0.192
Malathion	0.327	0.177–0.605	0.327
Nicotine	63.0	53.0–75.0	63.0
Parathion	0.0816	0.0685–0.0971	0.0816
Pyrenone	1.03	0.873–1.21	1.03
Pyrolan	0.302	0.255–0.359	0.302
Strobane	26.7	23.5–30.4	20.0
Isobornyl thiocyanoacetate	>28,000.0	—	>28,000.0

[a] Based on insects killed prior to completion of the pupal stage or before the emergence of morphologically normal adults.
[b] Based on insects in all stages killed between the time of treatment and 3 days after the emergence of adults. The total effect of direct and latent toxicity.

Table 3.—Summary of direct and total toxicity of several insecticides applied as soil treatments to larvae of *Dacus cucurbitae* (Coq.).

Insecticide	Direct Toxicity[a] LD-50 μg./gm.	Direct Toxicity[a] Fiducial Limits P=.05 μg./gm.	Total Toxicity[b] LD-50 μg./gm.
Aldrin	0.0188	0.00809–0.0437	0.0138
Chlordane	0.0945	0.0401 –0.222	0.0298
Dieldrin	0.0416	0.0292 –0.0591	0.0360
Endrin	0.0114	0.00504–0.0258	0.00838
Isodrin	0.00981	0.00465–0.0207	0.00665
Lindane	0.0339	0.0213 –0.0539	0.0339
Malathion	0.0489	0.0367 –0.0651	0.0489
Parathion	0.00520	0.00344–0.00787	0.00520

[a] Based on insects killed prior to completion of the pupal stage or before the emergence of morphologically normal adults.

[b] Based on insects in all stages killed between the time of treatment and 3 days after the emergence of adults. The total effect of direct and latent toxicity.

The total toxicity indices were: aldrin, 38; chlordane, 17; dieldrin, 14; endrin, 62; isodrin, 78; lindane, 15; and malathion, 11.

The relationship between the number of abnormal adults and latent toxicity was similar in this species to that in the Mediterranean fruit fly. Although there were some abnormal adults among the populations exposed to lindane, malathion, and parathion, their numbers were insignificant when compared with the other materials.

The latent toxic effect of the insecticides was not so great in the melon fly as in the Mediterranean fruit fly. This differential latent toxicity between the species may be explained by one or both of the following (Tamashiro & Sherman 1955):

1. The larvae, pupae, and adults of the melon fly detoxify the insecticide more readily than the corresponding stages of the Mediterranean fruit fly.

2. The Mediterranean fruit fly larva is much more resistant than the adult, but there is little difference in resistance between the melon fly larva and adult.

Ebeling (1953) found that Mediterranean fruit fly adults were less resistant than adult melon flies to space sprays of lindane, chlordane, aldrin, and dieldrin. This may account for the lack of latent toxicity of lindane to the melon fly. The adult Mediterranean fruit fly is less resistant to lindane than the adult melon fly, but the Mediterranean fruit fly larva is more resistant to lindane than the melon fly larva. Thus, it is quite possible that the carryover of lindane from the larval stage of the Mediterranean fruit fly may be sufficient to affect the adult stage, while the carryover in the melon fly is not great enough to affect the adult. Lindane, of course, showed a low order of latent toxicity even in the Mediterranean fruit fly.

It appears, therefore, that species, chemical structure, and latent toxicity are definitely interrelated. Probably, in those species where there is little difference in resistance between the immature and adult stages there would be little or no latent mortality exhibited. It would be worthwhile to investigate the susceptibility to this phenomenon of species of other holometabolous orders and also of heterometabolous species. The high order of latent toxicity was associated with the chlorinated polycyclic hydrocarbons.

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Laboratory and Field Tests with Sevin against Cotton Insects[1]

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Abstract

In laboratory tests at Brownsville, Tex., Sevin (1-naphthyl-N-methyl carbamate) applied as a spray to potted cotton plants was effective against the boll weevil (*Anthonomus grandis* Boh.), the pink bollworm (*Pectinophora gossypiella* (Saund.)), the bollworm (*Heliothis zea* (Boddie)), the cabbage looper (*Trichoplusia ni* (Hbn.)), and the cotton aphid (*Aphis gossypii* Glov.). On field plots in the Lower Rio Grande Valley, dusts containing 1.5 to 2.5 pounds of Sevin per acre applied at 4- to 7-day intervals gave control of the pink bollworm and boll weevil comparable with that obtained with standard insecticides.

Sevin (1-naphthyl-N-methyl carbamate)[3] was tested in the laboratory at Brownsville, Tex., in 1956 and early 1957 to compare its effectiveness with standard insecticides against various cotton insects. In the summer of 1957 it was tested in the field in the Lower Rio Grande Valley, primarily for the control of the boll weevil (*Anthonomus grandis* Boh.) and the pink bollworm (*Pectinophora gossypiella* (Saund.)), although records of aphid and spider mite infestations were also made.

Laboratory Tests.—In the laboratory tests the first formulations were made with technical Sevin in xylene, but later the manufacturer's 13% emulsifiable concentrate was used since it was much easier to keep in solution. The standard insecticides were dissolved in xylene to make stock solutions. Sprays were prepared by mixing a

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[3] Supplied by the Union Carbide Chemicals Company.

Table 1.—Comparative effectiveness of Sevin and standard insecticides used as sprays on cotton plants in laboratory tests against six insects.

Insecticide (Pounds per Acre)	Net Per Cent Mortalities After Indicated Hours of Exposure											
	Boll Weevil		Pink Bollworm		Bollworm		Cabbage Looper		Salt-Marsh Caterpillar		Cotton Leafworm	
	22	48	24	48	24	48	24	48	24	48	24	48
Sevin												
0.125	27	77	—	—	—	—	—	—	7	33	—	—
.25	47	95	11	35	27	33	10	20	13	40	25	35
.5	90	95	17	39	41	54	37	57	22	56	30	65
1	100	—	14	47	53	79	50	53	31	45	64	80
2	—	—	100	100	—	—	59	74	80	100	92	100
Dieldrin												
0.0625	40	77	—	—	—	—	—	—	—	—	—	—
.125	67	87	—	—	—	—	—	—	—	—	—	—
.25	87	100	—	—	—	—	—	—	—	—	—	—
.5	93	100	—	—	—	—	—	—	—	—	—	—
DDT												
0.25	—	—	14	9	14	50	—	—	—	—	—	—
.5	—	—	17	35	25	79	17	17	—	—	—	—
1	—	—	46	70	74	95	—	—	—	—	—	—
2	—	—	78	91	—	—	37	58	—	—	—	—
Endrin												
0.25	—	—	—	—	—	—	50	70	51	69	95	100
.5	—	—	—	—	73	73	73	73	55	85	—	—
1	—	—	—	—	—	—	73	87	83	97	—	—

sufficient amount of the stock solution or concentrate with distilled water to give the desired strengths; the water contained 1% of the emulsifier Triton X-151.

In one group of tests four concentrations of the insecticides were applied against several insects in order to determine dosage-mortality relationships. Immediately after the plants were sprayed, they were moved to the holding room where, usually within 1 hour, cages were placed over them and the test insects introduced. Greenhouse-grown potted cotton plants were used, and the holding room was maintained at 80° ± 2° F. and a relative humidity of 60 to 70%. Most of the tests were replicated three times. The insects included and the numbers of each species per test were as follows:

FIG. 1.—Cotton field at San Benito, Tex., that received 10 applications of 10% Sevin dust. The dark area at upper right was untreated.

Boll weevil (*Anthonomus grandis* Boh.)	15 to 30 adults
Pink bollworm (*Pectinophora gossypiella* (Saund.))	20 to 30 moths and 30 first-instar larvae
Bollworm (*Heliothis zea* (Boddie))	12 to 25 second- and third-instar larvae each
Cabbage looper (*Trichoplusia ni* (Hbn.))	
Salt-marsh caterpillar (*Estigmene acrea* (Drury))	
Cotton leafworm (*Alabama argillacea* (Hbn.)).	12 to 25 fifth-instar larvae

The average mortalities, after being corrected for natural mortality, resulting from these spray treatments are given in table 1, and the LD-50's in table 2.

In addition, residual life tests were made against the boll weevil and the pink bollworm by exposing them for 48 hours to residues of various ages—the boll weevil on glass plates and the pink bollworm on sprayed potted plants. The results of these tests are presented in table 3.

In the tests against the boll weevil, after the first 22 hours of exposure Sevin was less toxic than dieldrin, the LD-50 being only 0.27 as much as that of dieldrin. However, at the end of 48 hours 97% mortality had resulted from the 0.25-pound dosage of Sevin. These results indicate that Sevin is somewhat slower in action than diel-

Table 2.—LD-50's after 48 hours for Sevin and standard insecticides in tests reported in table 1.

Insect	Sevin	Standard Insecticide	
Boll weevil[a]	0.26	Dieldrin	0.07
Pink bollworm	.58	DDT	.68
Bollworm	.43	DDT	.25
Cabbage looper	.94	Endrin	.20
Salt-marsh caterpillar	.96	Endrin	.25
Cotton leafworm	.60	—	

[a] On basis of mortalities after 22 hours.

Table 3.—Per cent mortalities of adult boll weevils and pink bollworms after 48 hours' exposure to spray residues of various ages.

Insect and Insecticide (Pounds per Acre)	Age of Residues (Days)		
	2 or 3[a]	5	6
Boll weevils:			
Sevin 1	47	—	13
Dieldrin 0.5	60	—	7
Pink bollworms:			
Sevin 2	47	50	13
DDT 2	40	38	10

[a] 3 days for boll weevils, 2 days for pink bollworms.

Table 4.—Control of pink bollworm larvae on cotton plants sprayed in the laboratory with Sevin and DDT at 2 pounds per acre.

Insecticide	Number of Bolls	Number of Eggs Hatched	Mines		Larvae	
			Number per Boll	Per Cent Reduction	Number per Boll	Per Cent Reduction
Sevin	9	76	0.2	96	0.2	95
DDT	9	67	1.8	64	.7	83
Check	10	87	5.0	—	4.0	—

drin. The residual life of Sevin was similar to that of dieldrin. Although some insecticidal activity was indicated on the sixth day, the period of effectiveness of both insecticides appears to be limited to 3 or 4 days.

Against pink bollworm moths Sevin was similar to DDT when used at 0.25 and 0.5 pound per acre. At the 1-pound rate DDT caused over three times as much mortality as Sevin during the first 24 hours, but after 48 hours the mortality of moths exposed to DDT was only about one-third higher than of those exposed to Sevin. At the 2-pound rate Sevin caused 100% mortality in 24 hours, compared with 78% for DDT, and in 48 hours its relative effectiveness was 1.3 times that of DDT. The residual life of Sevin also appears to be similar to, or slightly longer than, that of DDT.

The toxicities of Sevin and DDT against pink bollworm larvae were compared by spraying the insecticides at the rate of 2 pounds per acre on potted cotton plants bearing one to three green bolls each. Bolls were infested by inserting slips of paper bearing 10 eggs ready to hatch under a bract of each boll. Table 4 shows the superiority of Sevin based on the reduction of both mines and larvae.

Table 6.—Control of pink bollworm and boll weevil with 10% Sevin dust in large plots near San Benito, Tex., and Rio Bravo, Mexico.

Treatment	Pounds of Toxicant per Acre	Pink Bollworm			Per Cent of Weevil-Punctured Squares	Yield of Seed Cotton (Pounds per Acre)	
		Per Cent of Infested		Number of Larvae per Boll		Total	Gain
		Blooms	Bolls				
San Benito							
Untreated	—	10.1	80.7	2.5	57.7	475	—
Treated	2.5	9.7	8.8	0.1	8.7	1880	1405
Rio Bravo							
Untreated	—	22.5	57.5	1.6	—	1260	—
Treated	3.2	4.0	3.0	0	—	2940	1680

Against bollworm larvae Sevin at 1 pound per acre caused 79% mortality, which is slightly higher than that from endrin and the same as that from DDT at 0.5 pound per acre. Its relative effectiveness was 0.58 that of DDT at LD-50.

Against cabbage looper larvae 2 pounds per acre gave 74% mortality in 48 hours compared with 58% from the same dosage of DDT and 87% from 1 pound of endrin.

Against the salt-marsh caterpillar the toxicity of Sevin was only 0.27 that of endrin at LD-50. However, the data indicate that a dosage somewhere between 1 and 2 pounds per acre would be effective.

In tests with cotton leafworm larvae, Sevin at 1 pound per acre killed 80% in 48 hours, and at 2 pounds killed 92 and 100% in 24 and 48 hours, respectively. Endrin at 0.25 pound per acre killed 95% in 24 hours.

In a single test against the cotton aphid (*Aphis gossypii* Glov.), Sevin at 0.0015% in acetone sprayed on cotton seedlings infested with all stages of the insect killed 56% in 24 hours and 67% in 48 hours, as compared with 70 and 83% respectively, when schradan was used at a concentration of 0.125%.

In a similar test against the two-spotted spider mite (*Tetranychus telarius* (L.)), a 1% concentration of Sevin gave 81% kill in 24 hours and no increase after 48 hours, whereas the respective kills with schradan were 46 and 71%.

No phytotoxicity to cotton has been observed from any concentration of Sevin that was used in these tests.

In these laboratory tests Sevin compared favorably with the insecticides now recommended for control of the boll weevil, pink bollworm, bollworm, and cabbage

Table 5.—Control of cotton insects with insecticide dusts in small-plot field experiment at San Benito, Texas.

Insecticide (Per Cent)	Boll Weevil	Bollworm		Pink Bollworm		Aphids per Leaf	Spider Mites per Leaf	Yield of Seed Cotton (Pounds per Acre)	
	Per Cent of Punctured Squares	Per Cent of Injured Squares	Per Cent of Injured Bolls	Per Cent of Infested Bolls	Number of Mines per Boll			Total	Gain
Sevin 2.5	38.9	3.2	1.7	65	3.5	0.1	0.16	545	395
5	16.3	0.8	0	35	0.8	0	.02	1096	946
10	6.3	1.5	0	5	.1	0	0	1195	1045
Guthion 3 plus DDT 5	11.8	3.0	0	8	.1	.2	0	1092	942
Dieldrin 2.5 plus DDT 10 plus sulfur 40	8.5	0.8	0	14	.3	0	.03	1260	1110
Untreated	66.5	18.7	5.5	87	7.6	15.6	.71	150	—

looper. Against the salt-marsh caterpillar it was less effective than endrin at the lower dosages but was effective when used at 2 pounds per acre. Against the cotton leafworm it was effective at 1 pound. Although Sevin cannot be considered one of the most effective acaricides or aphidicides, it may have enough activity against these pests when used to control other cotton insects to prevent damaging populations from building up.

FIELD TESTS.—Two series of field tests with Sevin dusts were conducted—one on small (0.2 acre) replicated plots in a field of late-planted irrigated cotton near San Benito, Tex., and the other on large unreplicated plots near San Benito and at Rio Bravo, Mexico.

In the small plots three concentrations of Sevin were compared with mixtures of DDT plus Guthion and DDT plus dieldrin and sulfur for control of the pink bollworm, boll weevil, aphids, and spider mites. The plots were arranged in a randomized block, and each treatment was replicated four times. In order to meet the August 31 plow-up deadline, the plants were chemically defoliated before many of the bolls had matured, which of course reduced the yield. Eleven applications, each of approximately 22 pounds of dust per acre, were made at 4- and 5-day intervals between June 28 and August 10. Three pink bollworm inspection counts 8 to 10 days apart were made beginning 4 weeks after the first application. One boll weevil count was made prior to and five following the first application. Aphid and spider mite infestations were determined from five counts of each during the period of the experiment.

As shown in table 5, the 5% and 10% Sevin dusts compared favorably with the Guthion-DDT and dieldrin-DDT-sulfur in the reductions of punctured squares, damaged bolls, aphid and mite populations, and also in the gains in yield. The 2.5% Sevin dust was relatively ineffective.

In the large plots a 10% Sevin dust was tested for control of the pink bollworm and the boll weevil. Applications were made with hand guns at 4- to 7-day intervals, 10 at San Benito and 12 at Rio Bravo. Most of the treatments were made under adverse weather conditions. One application at Rio Bravo was washed off by rain. One application of sulfur to control spider mites was made on the Sevin test plot near San Benito, and on the untreated area near Rio Bravo. One bloom-inspection count for the pink bollworm was made prior to the first application, and six boll-infestation counts on each plot after dusting was started. High infestations developed in both plots shortly after the first blooms appeared and before the first poison application. A damaging boll weevil infestation developed in San Benito toward the latter part of the season but not at Rio Bravo.

Very good insect control was obtained with Sevin, as is reflected in the excellent increases in yield (table 6). Its effectiveness in controlling the insects in the test at San Benito is also shown in figure 1.

Insect Survival in Jet Aircraft[1,2]

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ABSTRACT

To find out if insects will survive the low temperatures, reduced pressure, and low humidity in the stratosphere where jet aircraft operate, several species were exposed in the unpressurized and essentially unheated sections of jet fighters and bombers during flights. A high mortality was obtained in 45- to 55-minute high-level flights of jet fighters except in the radar compartment, where heat from electronic equipment gave protection. In a jet bomber all the insects were killed on a 3-hour flight at 40,000 feet (outside air temperature −51° C.) and in two of three flights of 2 to 2½ hours at 35,000 to 40,000 feet. Shorter flights at that altitude or long flights at low altitudes gave reduced kills.

The development of jet aircraft, which operate in the stratosphere, raised the question as to whether insects can survive the high altitudes, and, if not whether present disinfestation practices on planes are in need of modification. Between 36,000 and 66,000 feet the standard day temperature is −69° C., the atmospheric pressure is one-fifth that at sea level, and the humidity is very low. Early in 1957 tests to answer this question were conducted at the U. S. Naval Air Station at Jacksonville, Fla. Jet fighters and bombers were used, because they are generally unpressurized and unheated except for the small pilot's compartment.

Jet bombers are normally parked with the bomb bay and cockpit access way opened. Tail, radar, and compartments at the side or forward of the cockpit are usually closed, being opened only when work is being performed in them. However, insects could gain entry while work is being done. There is less danger in fighter aircraft, but insects may be introduced with ammunition that has been lying around in infested areas or when the compartments are opened for inspection.

In previous laboratory tests with a low-temperature cabinet Knipling & Sullivan (1957) found strong indications that many insects would be killed if exposed for 1 hour to temperatures between —15° and −30° C. House flies and yellow-fever mosquitoes were killed when exposed for 30 minutes or longer at −20°. In the present study the insects were tested under flight conditions.

Insects were exposed in the ammunition, radar, and nose-gear access-well compartments of FJ3 (Fury) fighter aircraft during four flights, each by two planes flying in formation. Yellow-fever mosquitoes (*Aedes aegypti* (L.)), house flies (*Musca domestica* L.), German cockroaches (*Blattella germanica* (L.)), American cockroaches (*Periplaneta americana* (L.)), and confused flour beetles (*Tribolium confusum* Duv.) were placed in wire-screen cages, and oriental rat fleas (*Xenopsylla cheopis* (Rothsch.)) in glass vials with cloth tops. Approximately

[1] Accepted for publication November 12, 1957.

[2] The opinions or assertions contained herein are the private ones of the writers and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large.

Table 1.—Temperature of the outside air and time at various altitudes during test flights in jet fighters and bomber aircraft.

Flight No.	Air Temperature		Time	
	1000 Feet	° C.	1000 Feet	Minutes
		In Fighters		
1	40	−61	40–45	40
	35	−51	30–39	10
	25	−28	<30	40
2	40	−52	40	30
	35	−49	33	15
	30 to 20	−43 to −22	<33	55
3	40	−63	43	20
	35	−50	26	35
	30	−38	26 to 20	20
	25 to 15	−25 to −7	<20	20
4	30	−43	30	10
	15	− 9	15	50
		In Bomber Aircraft		
1	40	−51	40	180
	—	—	2.5 to 5	60
2	25	−27	40	30
	—	—	35	120
3	40	−51	37	150
	35	−45	10	60
4	40	−51	39	120
	—	—	5 to 15	80
5	25	−27	37	40
	20	−16	23	185
6	25	−18	23	150
	20 to 5	−17 to +7	Low	20
7	35	−39	32	40
	30	−32	Low	140
	25 to 5	−18 to +7		
8	Same as No. 7		15	10
			Low	100
9	Same as No. 7		5 to 2.5	130

600 insects were used in each aircraft. The test and check insects, which were kept under similar conditions except for the flights, were unfed during the test period. All insects were then fed and held in the laboratory for a 24-hour observation period.

The flight and insect-mortality data for the four flights are given in tables 1 and 2. All insects in the ammunition and nose-gear well compartments were killed in all flights of 45 to 55 minutes at 26,000 to 45,000 feet, with one exception; an 85% control of the flour beetles resulted when they were protected by several layers of canvas. In the radar compartment the kill varied between 0 and 100%, depending on the heat given off by the electronic equipment. This compartment is not normally opened except for repairs. A flight of 10 minutes at 30,000 feet was not long enough to kill insects.

Nine flights were made in which the same species were exposed in unpressurized compartments of A3D (Skywarrior) bomber aircraft (figure 1), except that the common malaria mosquito (*Anopheles quadrimaculatus* (Say)) was substituted for the American cockroach. These compartments comprised about 1,700 cubic feet of the plane's total volume of approximately 2,000 cubic feet, since only the pilot's compartment is pressurized and heated. All compartments except the bomb bay, companion way, and right and left forward compartments contained electronic equipment.

Table 2.—Per cent mortality of insects after flights in unpressurized compartments of jet fighter aircraft.[a]

Compartment and Insect	Flight 1		Flight 2		Flight 3		Flight 4	
	A	B	A	B	A	B	A	B
Radar								
Yellow-fever mosquito	—	—	100	100	100	100	33	42
House fly	—	100	0	100	100	100	—	—
Confused flour beetle	—	100	—	—	10	100	—	0
German cockroach	100	100	—	—	—	100	—	0
Oriental rat flea	—	—	0	100	—	98	—	—
Nose-Gear Access Well								
Yellow-fever mosquito	100	—	100	—	100	—	27	—
House fly	—	—	100	100	100	—	—	—
Confused flour beetle	100	—	—	100	100	—	—	—
American cockroach	—	100	—	—	—	—	—	—
Oriental rat flea	—	—	—	—	100	—	0	—
Ammunition								
Yellow-fever mosquito	100	—	100	100	100	100	5	26
House fly	—	100	100	100	100	—	—	—
Confused flour beetle	100	—	100	100	85[b]	100	—	0
German cockroach	100	—	—	—	100	100	—	10
American cockroach	100	—	—	—	—	—	—	—
Oriental rat flea	—	—	100	—	100	—	5	—

[a] A and B are paired planes.
[b] Protected by several layers of cloth.

The results of these tests are given in table 3. All insects were killed on a 3-hour flight at 40,000 feet (outside air temperature −51° C.) and in two of three flights of 2 to 2½ hours at 35,000 to 40,000 feet. In the third flight

Table 3.—Per cent mortality of insects after flights in unpressurized compartments of jet bomber aircraft.

Compartment and Insect	Flight Number								
	1	2	3	4	5	6	7	8	9
Forward Left									
Yellow-fever mosquito	—	—	—	—	—	—	—	—	3
Malaria mosquito	100	—	—	100	—	—	—	—	—
House fly	100	—	—	100	—	100	—	—	5
Confused flour beetle	100	—	—	100	—	100	—	4	0
Oriental rat flea	—	—	—	100	—	—	—	—	—
Forward Right									
Yellow-fever mosquito	—	—	—	—	100	—	—	17	—
Malaria mosquito	—	—	90	100	—	—	—	—	10
House fly	—	—	100	100	100	—	85	—	0
Confused flour beetle	—	—	100	100	55	—	70	9	100
German cockroach	—	—	100	100	—	—	—	—	—
Oriental rat flea	—	—	100	—	—	—	—	—	—
Companionway									
Yellow-fever mosquito	—	100	—	—	—	—	—	—	—
House fly	—	100	—	—	—	5	—	—	—
Bomb Bay									
Yellow-fever mosquito	—	100	—	—	68	48	50	55	40
Malaria mosquito	100	—	—	100	—	—	—	15	—
House fly	100	100	85	100	0	30	8	—	0
Confused flour beetle	100	100	10	100	53	25	0	14	1
German cockroach	100	—	0	100	—	—	—	—	—
Oriental rat flea	—	—	95	100	—	—	—	—	—
Radar									
Yellow-fever mosquito	—	—	—	—	100	100	—	69	—
Malaria mosquito	100	—	100	—	—	—	—	15	—
House fly	—	—	—	100	0	100	—	—	—
Confused flour beetle	100	—	100	100	100	100	100	8	0
German cockroach	100	—	100	100	—	—	—	—	—
Oriental rat flea	—	—	100	—	—	—	—	—	—
Tail									
Yellow-fever mosquito	—	100	—	—	100	100	100	50	90
Malaria mosquito	100	—	—	100	—	—	—	10	—
House fly	100	100	100	100	35	100	—	—	0
Confused flour beetle	—	—	100	100	100	92	100	5	0
German cockroach	100	—	—	100	—	—	—	—	—
Oriental rat flea	—	—	100	—	—	—	—	—	—

FIG. 1.—U. S. Navy A3D (Skywarrior) jet bomber used to determine the effects of high altitudes on insects in unpressurized compartments. (Official photograph U. S. Navy.)

some insects survived in the bomb bay owing to heat generated from the air-driven unit. This situation could be remedied by "cracking open" the bomb-bay doors for a few minutes during flight. Some insects survived in tool boxes and canvas bags in the bomb bay, perhaps because heat transfer through the air is poor in the rare atmosphere of the stratosphere. Flights of 40 minutes or less in the stratosphere or long flights at lower altitudes gave unsatisfactory kills. The mortality of check insects was low.

From these tests it is tentatively concluded that the cold in the stratosphere gives a high insect kill in unheated compartments of jet aircraft during long flights. However, further tests should be made by exposing insects to sustained flight conditions in various types of jet bombers and fighters and attaching egg masses to the outside "skin" of the aircraft. It is considered that baggage and cargo affording protection to insects would require inspection or other appropriate quarantine measures.

REFERENCE CITED

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Resistance Tests with the Bed Bug and Evaluation of Insecticides for Its Control[1]

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ABSTRACT

Resistance to DDT was demonstrated in bed bugs (*Cimex lectularius* L.) from Japan, Korea, Ohio, and two naval vessels. A continuous exposure test for determining the presence of resistance is described. In laboratory tests residues of malathion, Am. Cyanamid 4124, and synergized pyrethrins were effective for the longest periods. Diazinon gave the fastest reduction of natural infestations. Dow ET-57 (sampled as ET-14), Dipterex, Chlorthion, malathion, dieldrin, and DDT gave slower but complete control. Synergized pyrethrins gave complete control in 2 days, but newly hatched nymphs were present after 1 week.

Since World War II, DDT has been used extensively for the control of bed bugs. Reports of the failure of DDT to control natural infestations of bed bugs, *Cimex lectularius* L. and *C. hemipterus* F., in Hawaii, Greece, Israel, Italy, and China have been collected by Brown (1956). Laboratory confirmation of *lectularius* resistance to DDT has been made from Teheran, Iran, by Mofidi & Samimi (1956). *C. hemipterus* from an army camp in Feng-Shan, Formosa, has been reported resistant by Chen *et al.* (1956) and from India (Bombay State) by Ramachandra Rao & Halgeri (1956). Cwilich *et al.* (1957) demonstrated a high degree of resistance to gamma-BHC in *lectularius* in Israel.

This paper describes work done at Orlando, Fla., on the determination of resistance in several strains of bed bugs and laboratory and field evaluations of several insecticides as substitutes for DDT.

[1] Accepted for publication November 7, 1957.

Table 1.—Relative susceptibility of several strains of bed bugs to DDT residues on wool cloth.

Strain	Per Cent Kill After Indicated Exposure Time							
	15 Minutes		30 Minutes		60 Minutes		120 Minutes	
	In 24 Hours	In 48 Hours	In 24 Hours	In 48 Hours	In 24 Hours	In 48 Hours	In 24 Hours	In 48 Hours
Kuskoa	0	0	0	0	0	0	0	0
U.S.S. *Salerno Bay*	0	0	0	0	0	10	0	0
Columbus	0	10	0	10	0	0	0	0
Korea	20	40	40	50	40	40	30	50
Orlando	40	60	80	100	80	80	100	100
Laboratory	30	30	40	70	70	80	50	90

Resistance Studies.—In 1953 tests were made with four strains of bed bugs from areas where DDT was not giving satisfactory control. These collections were made by the U. S. Army in Pusan, Korea, and in Yokosuka, Japan (Kuskoa strain); by the U. S. Navy on the U.S.S. *Salerno Bay*; and by J. J. Mooney in Columbus, Ohio. The Korean strain, collected in 1951, had been reared in the laboratory for 2 years with no exposure to insecticides. A strain collected in a house in Orlando, Fla., and the normal laboratory colony were included for comparison.

The tests were conducted on round wool patches, 4 inches in diameter, impregnated with a 2% solution of DDT in acetone. After the patches had dried, they were placed in petri dishes, and duplicate sets of five bugs each were exposed on them for periods ranging from 15 to 120 minutes. The bugs were then transferred to clean petri dishes, and mortality counts were taken after 24 and 48 hours.

The results are presented in table 1. Bed bugs from the *Salerno Bay*, Columbus,[2] and Kuskoa strains were very resistant to DDT, exposures of 15 to 120 minutes causing no more than 10% mortality in 48 hours. The Korean strain was slightly resistant, whereas the Orlando field-collected strain was a little more susceptible than the laboratory colony.

In other tests only 20% of the Columbus bed bugs and 22% of those from U.S.S. *Salerno Bay* were killed by a 1-week exposure on the DDT-treated cloth.

Tests were also run to determine the susceptibility of the Kuskoa strain to lindane, chlordane, and methoxychlor and of the Columbus bugs to dieldrin and aldrin. Wool patches were impregnated with 2% solutions of the insecticides. Some bugs were exposed on the patches for 2 hours, and the mortality recorded after 24 and 48 hours; others were exposed continuously for periods up to 1 week, and the mortality recorded every 24 hours.

Table 2.—Susceptibility of DDT-resistant bed bugs to several chlorinated hydrocarbon insecticides.

Insecticide	Per Cent Mortality After Exposure for—			
	2 Hours		Continuous	
	24 Hours	48 Hours	24 Hours	48 Hours
	Kuskoa Strain			
Lindane	100	100	100	100
Chlordane	0	40	70	100
Methoxychlor	0	0	0	0
	Columbus Strain			
Dieldrin	0	60	0	100
Aldrin	0	20	70	100

The results are given in table 2. Lindane was the most effective insecticide, a 2-hour exposure causing 100% mortality in 24 hours. Chlordane, dieldrin, and aldrin were relatively ineffective at a 2-hour exposure, but a 48-hour exposure caused 100% mortality. Methoxychlor was completely ineffective, causing no mortality after continuous exposure for 168 hours.

In April 1956 three collections of bed bugs (Stanton, Johns, and Gillette) were made from natural infestations in tenements in Denver, Colo. The colonies were reared in the laboratory until sufficient numbers were obtained for testing. They were compared with bugs from the normal laboratory colony for susceptibility to DDT by continuous exposure on filter paper and glass surfaces. The procedure was as follows: Strips of Whatman No. 2 filter paper, $1\frac{1}{2}$ by $1\frac{1}{4}$ inches, were impregnated with 0.2 ml. of a 1% DDT solution in acetone. At the same time 0.2 ml. of the same solution was pipetted onto the bottom of a 50-ml. beaker, which has about the same area as the filter paper. When dry, the filter papers were placed vertically in the treated beakers with the longer dimension across the diameter. Ten bed bugs were placed in each beaker, and if balled up were separated. They soon crawled onto the filter paper and only occasionally returned to the glass surface. Exposure was continuous, and knockdown counts were made at various intervals ranging from 12 to

[2] Results of some tests with this strain were summarized anonymously in Soap and Sanit. Chem. 29(8): 171 (1953).

Table 3.—Susceptibility of four strains of bed bugs to continuous exposure to residues of DDT on filter paper and glass surfaces.

Strain and Days Since Last Blood Meal	Per Cent Knockdown and Kill After—(Hours Shown)							Calculated Lethal Time (Hours)	
	12	24	32	40	48	68	84[a]	LT-50	LT-90
Gillette									
1	17	30	—	63	70	87	100	31.5	85.4
3	23	67	90	—	100	—	—	17.2	36.5
Johns									
1	10	20	—	23	23	73	100	56.3	89.8
3	13	50	67	—	100	—	—	21.8	36.5
Stanton									
1	7	7	—	40	40	87	100	45.0	86.7
3	37	83	87	—	100	—	—	13.9	34.5
Normal									
1	20	20	—	83	83	100	—	26.5	56.1
3	37	80	87	—	100	—	—	13.7	36.6

[a] Mortality in untreated checks: Gillette 10% (3-day starved), normal 3%

Table 4.—Susceptibility to lindane and DDT of a collection of bed bugs from U.S.S. *Valley Forge* and a normal strain.

Strain	Number of Bugs	Per Cent Knockdown and Kill After—							Calculated Lethal Time (Hours)	
		Hours								
		10	14	18	36	60	84	108	LT-50	LT-90
DDT:										
Normal	30	30	37	47	97	100	—	—	16.1	27.8
Valley Forge	10	10	10	10	30	70	90	100	39.5	107.3
		Minutes								
		45	50	60	70	80	90			
Lindane:										
Normal	15	7	13	47	73	80	100		1.05	1.41
Valley Forge	13	8	23	54	69	69	100		1.06	1.55

Table 5.—Susceptibility of normal bed bugs to residues of various insecticides on wool patches.[a]

Insecticide and Age of Residue	Per Cent Kill After Exposure for—					
	½ Hour		2 Hours		24 Hours	
	In 24 Hours	In 96 Hours	In 24 Hours	In 96 Hours	In 24 Hours	In 96 Hours
DDT:						
2 hours	25	50	40	65	100	—
1 week	60	95	35	90	80	100[b]
2 weeks	0	75	50	100	60	100[c]
4 weeks	—	—	40	70	50	100[b]
8 weeks	—	—	50	95	75	100[b]
12 weeks	—	—	10	70	35	95
16 weeks	—	—	5	30	50	100
20 weeks	—	—	—	—	70	95
24 weeks	—	—	—	—	55	100[c]
28 weeks	—	—	—	—	15	70
Chlordane:						
2 hours	45	55	100	—	100	—
1 week	15	70	85	100[c]	95	100[b]
2 weeks	0	15	0	50	100	—
8 weeks	—	—	25	80	100	—
12 weeks	—	—	0	25	80	90
16 weeks	—	—	—	—	45	95
20 weeks	—	—	—	—	45	70
24 weeks	—	—	—	—	15	25
Dieldrin:						
2 hours	20	70	45	100	100	—
1 week	50	85	75	100	100	—
2 weeks	10	95	15	75	100	—
4 weeks	—	—	65	100	100	—
12 weeks	—	—	50	100	100	—
16 weeks	—	—	50	95	100	—
24 weeks	—	—	65	85	100	—
28 weeks	—	—	10	35	100	—
Lindane:						
8 weeks	100	—	100	—	100	—
12 weeks	95	95	100	—	100	—
16 weeks	0	15	80	85	100	—
20 weeks	—	—	5	15	45	50
Malathion:						
2 hours	100	—	100	—	100	—
1 week	75	80	100	—	100	—
2 weeks	100	—	100	—	100	—
4 weeks	90	95	95	95	100	—
8 weeks	100	—	100	—	100	—
20 weeks	95	95	100	—	100	—
24 weeks	100	—	100	—	100	—
28 weeks	0	5	35	40	100	—
Am. Cyanamid 4124:						
8 weeks	100	—	100	—	100	—
12 weeks	95	95	100	—	100	—
16 weeks	100	—	100	—	100	—
20 weeks	40	65	75	75	100	—
24 weeks	15	15	50	55	100	—
28 weeks	—	—	0	0	50	60
Chlorthion:						
1 week	100	—	100	—	100	—
2 weeks	90	90	90	100[c]	100	—
8 weeks	85	90	95	95	100	—
12 weeks	20	30	50	55	100	—
16 weeks	—	—	—	—	75	85
20 weeks	—	—	—	—	0	0

Table 5.—(continued)

Insecticide and Age of Residue	Per Cent Kill After Exposure for—					
	½ Hour		2 Hours		24 Hours	
	In 24 Hours	In 96 Hours	In 24 Hours	In 96 Hours	In 24 Hours	In 96 Hours
Diazinon:						
4 weeks	100	—	100	—	100	—
8 weeks	55	60	100	—	100	—
12 weeks	0	5	15	35	100	—
20 weeks	—	—	—	—	100	—
24 weeks	—	—	—	—	75	90
28 weeks	—	—	—	—	5	5
Dipterex:						
2 hours	100	—	100	—	100	—
1 week	0	0	35	50	100	—
2 weeks	5	30	0	30	45	70
4 weeks	—	—	—	—	45	75
8 weeks	—	—	—	—	5	15
Dow ET-57:						
1 week	100	—	100	—	100	—
2 weeks	90	100	85	100[c]	100	—
4 weeks	100	—	100	—	100	—
8 weeks	0	80	80	100	100	—
12 weeks	0	0	0	10	25	80
16 weeks	—	—	—	—	15	75
20 weeks	—	—	—	—	0	5
Pyrethrins 0.4%+piperonyl butoxide 4%:						
8 weeks	100	—	100	—	100	—
16 weeks	95	100	100	—	100	—
20 weeks	100	—	100	—	100	—
24 weeks	100	—	100	—	100	—
28 weeks	85	85	100	—	100	—

[a] Check mortality never exceeded 5% after 24 hours or 10% after 96 hours
[b] 100% after 48 hours.
[c] 100% after 72 hours.

84 hours. The bed bugs were considered knocked down when they could no longer hold onto the filter paper. They were tested on the first or third day after their last blood meal, and all tests were run in triplicate. Both male and female bugs were used, since other tests had shown no appreciable difference in the susceptibility. The knockdown and mortality counts and the estimated LT-50's and LT-90's are recorded in table 3.

None of the strains showed any appreciable resistance to DDT. All were more susceptible on the third day than on the first day after feeding.

In 1957 a collection of bed bugs from the U.S.S. *Valley Forge* was tested for their susceptibility to continuous exposure on DDT and lindane residues on glass and filter paper by the method described above. Two-tenths of a milliliter of 1.0% DDT or 0.05% lindane was applied to both filter paper and beaker bottom. The bugs were

Table 6.—Control of natural populations of bed bugs in substandard hotels with several insecticides.

Insecticide and Concentration (%)	Number of Rooms	Number of Live Bed Bugs Found After—(Days)							
		1	2	3–5	7–8	9–10	11	12	14
Diazinon									
0.5	4	0	0	0	—	0	0	—	—
.1	5	1	1	1	—	0	0	—	—
Dow ET-57									
1.0	4	6	0	1	0	0	0	1	0
.5	4	1	3	1	0	0	0	0	0
Dipterex									
1.0	5	13	6	0	0	0	0	0	0
.5	4	23	14	0	8	—	0	0	0
Chlorthion									
1.0	4	2	5	0	0	—	0	0	0
.5	4	1	0	11	1	—	0	0	0
Malathion									
1.0	5	3	1	3	0	0	0	0	0
.5	6	1	0	0	1	0	0	0	0
Dieldrin									
.5	3	5	7	5	—	0	0	0	—
DDT									
5.0	3	41	4	4	1	0	1	0	—
Pyrethrins plus piperonyl butoxide 0.1+1.0	3	11	0	0	—[a]	—[a]	—	—	—

[a] Newly hatched nymphs in all treated rooms.

exposed on the third day after their last blood meal. The results are given in table 4. The *Valley Forge* strain required an exposure to DDT two to three times longer than the normal colony. The two strains were equally susceptible to lindane.

LABORATORY EVALUATION OF INSECTICIDES.—The residual effectiveness of 11 selected insecticides was determined in laboratory tests. Circular wool cloth patches, 1½ inches in diameter, were treated with 0.7 ml. of a 0.4% acetone solution of the insecticide, dried, and placed in 50-ml. beakers. Normal adult bed bugs that had been starved for 3 days were exposed on the patches in duplicate sets of 10 each for periods of ½, 2, and 24 hours. Mortality counts were made 24 hours after the beginning of the exposure period and on each subsequent day for 4 days. The patches were retested after aging for 1 to 28 weeks.

The results of the tests are recorded in table 5. Malathion and pyrethrins plus piperonyl butoxide were the most effective materials, a half-hour exposure giving 100% kill after 24 weeks. Based on the 2-hour exposure, the other insecticides, in descending order of effectiveness, were Am. Cyanamid 4124, lindane, dieldrin, Diazinon, Chlorthion, Dow ET-57 (sampled as ET-14), chlordane, and Dipterex. DDT was less effective initially than chlordane or Dipterex but more effective after the first 2 weeks.

TESTS IN HOMES, ROOMS, AND APARTMENTS.—Eight insecticides were evaluated for bed bug control in rooms and apartments in substandard hotels in Denver, Colo. One to two pints of odorless kerosene solutions were applied with a compressed-air sprayer to bed frames, mattresses, and woodwork in each infested room. Each mattress was sprayed until it had a frosty appearance, which usually required about ½ pint. The tests were evaluated on the basis of the number of live bed bugs found during inspections made at night over a 2-week period.

The results are given in table 6. No live bed bugs were found in the rooms treated with 0.5% of Diazinon throughout 11 days of observation, and only one was found at each of the first three inspection periods in five rooms treated with 0.1%. With Dow ET-57, Dipterex, Chlorthion, malathion, dieldrin, and DDT 2 to 8 days were usually required to achieve complete control. Rooms treated with pyrethrins plus piperonyl butoxide were free of bed bugs after 2 days, but were reinfested with newly hatched nymphs after about 1 week.

A second series of tests was conducted in houses in Orlando, Fla., comparing malathion and Am. Cyanamid 4124. The application procedure was the same as in the Denver tests. The homes were inspected for live bed bugs over a 9-month period. Two days after treatment one live bed bug was found in each of two homes treated with malathion and in two of five homes treated with Am. Cyanamid 4124. No live bed bugs were found in any of the homes during the remainder of the inspection period.

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NOTICE TO AUTHORS

Each manuscript submitted for publication in the JOURNAL OF ECONOMIC ENTOMOLOGY must be accompanied by an abstract suitable for publication, and prepared in accordance with instructions for the preparation of abstracts as required by *Biological Abstracts*. This abstract will replace the usual author's summary. See "Important Notice to Authors" which appeared in the *Bulletin of the Entomological Society of America* 3(2): 1. June, 1957.

Thimet Soil Treatments for the Control of Leaf Miners[1]

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ABSTRACT

Control of the birch leaf miner (*Fenusa pusilla* (Lep.)), the holly leaf miner (*Phytomyza ilicis* (Curt.)), and the boxwood leaf miner (*Monarthropalpus buxi* (Lab.)) was obtained with 2% Thimet granules sifted onto the surface of the ground under the plants as far as their periphery. With the exception of small holly plants, the granules were not worked into the soil. In most instances watering was done to facilitate quick absorption of the systemic by the plants. April treatments gave good control of the first two broods of birch leaf miners, whereas June treatments were not so effective. Holly leaf miner was effectively controlled in large trees with 4 to 16 oz. and in small trees with 1 to 2 oz. of Thimet. Most boxwood leaf miners were destroyed with 0.08 to 0.32 oz. and somewhat less effective results were obtained with 0.01 to 0.005 oz. of Thimet per average 22-inch plant.

During the season of 1957, experiments were conducted to evaluate Thimet as a soil systemic for the control of the birch leaf miner(*Fenusa pusilla* (Lep.)), the holly leaf miner (*Phytomyza ilicis* (Curt.)) and the boxwood leaf miner (*Monarthropalpus buxi* (Lab.)). All three species are serious pests of their respective host plants. In the past, the use of insecticides as foliage sprays has imposed the problem of critical timing for best results in control (Schread 1954). More recently Cory & Highland (1957) found that the systemic insecticides, Systox and schradan used as soil or trunk treatments produced effective control of boxwood and holly leaf miners. In addition, Thimet used as a foliage spray and as a soil drench has given good control of the iris borer, *Macronoctua onusta* Grote (Schread 1957).

MATERIAL AND METHODS.—Granules containing 2% of Thimet were used in all of the experiments. The formulation used in the birch leaf miner experiments was made in 1956. The material used in the holly and boxwood leaf miner experiments was formulated in 1957. Treatments were applied to the surface of the ground. With one exception it was not worked into the soil. The exception was in the control of the holly leaf miner in small plants growing in nursery rows where the ground was loose allowing Thimet to be raked into the soil to a depth of ½ to 1 inch. Because of the extended drought most of the treatments were watered in at the time of application and subsequently at intervals of 5 to 10 days for the remainder of the summer.

CONTROL OF THE BIRCH LEAF MINER.—On April 12, granules containing 2% of thimet were sifted onto the soil under clumps of gray birch trees at the rate of 22 or 40 pounds of the active ingredient per acre. The areas treated included all of the ground under the trees to the periphery of each clump. One clump of trees was used per treatment. The soil was hard packed clay. The treatments were designed to control the first and second broods of miners.

A second series of treatments as made on June 4 on additional trees to control the second brood occurring in late June and early July. In these tests the granules were used at the rate of 20, 10, 5 or 2.5 pounds of actual Thimet per acre. The trees varied in height from 6 to 18 feet with an average of three trees per clump.

RESULTS.—Both dosages applied on April 12 gave excellent control of the first brood of miners. The eggs apparently hatched but the larvae were killed before they fed very much. Dissections showed that all the larvae were dead in treated trees. The April treatment also gave complete control of the second generation. Almost all of the miners in untreated trees of the first test had completed their development and emerged before the data on control were taken on June 3. Hence, only seven live larvae were found in the 50 leaves examined from the controls. An average of 85.6% of the areas of these leaves had been mined.

Results of an attempt to control the second brood of the birch leaf miner are given in table 1. The data presented indicate that an early June treatment with 2% Thimet granules did not give as good control of the second brood as an April treatment gave of the first brood. Many larvae completed their feeding and emerged in the usual manner. These are included with the live miners in figuring per cent kill. Because of the way in which the leaves were mined it was not always possible to determine the exact number of miners that had actually emerged from injured foliage. Therefore, only an estimate is given. The percentage of leaf surface mined was likewise estimated. The treatments were not watered down at time of application, but because of serious drought one irrigation was applied June 19.

CONTROL OF HOLLY LEAF MINER.—Adults of the 1956 brood of the holly leaf miner began emerging on May 21, 1957 in the vicinity of New Haven, and 80% of the adults emerged during the following week .Egg laying in the new leaves occurred during this time. The new brood of leaf miners continued normal development for the next 6 weeks. At the end of this time (July 10) 2% Thimet was applied as a side dressing to 12- to 24-inch holly plants

Table 1.—Control of the second brood of the birch leaf miner with 2% Thimet granules.[a] 1957.

THIMET (POUNDS PER ACRE)	AVERAGE NO. STINGS PER LEAF	AVERAGE PER CENT OF LEAF SURFACE MINED[b]	LEAF MINERS			PER CENT KILL
			Alive	Dead	Emerged[b]	
2.5	2.4	24.3	30	19	77	15.0
5.0	3.17	29.1	51	58	64	33.5
10.0	3.2	28.0	32	23	25	28.7
20.0	2.8	23.4	28	29	23	36.2
Untreated	2.8	32.6	32	1	32	—

[a] Treatment applied June 4; results based on 50-leaf samples taken July 10.
[b] Estimated.

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Table 2.—Control of the holly leaf miner with 2% Thimet granules. 1957.

Ounces of Thimet per Tree	Average Number Mines per Leaf	Leaf Miners		Per Cent Kill
		Alive	Dead	
	Treated July 10			
1	2.2	20	70	77.7
2	1.9	3	73	96.0
Untreated		124	1	
	Treated July 19			
4	3.6	2	143	98.6
8	1.9	11	68	86.0
12	3.2	7	123	94.6
16	2.4	0	97	100.0
Untreated	2.4	99	0	—

Table 3.—Control of the boxwood leaf miner with 2% Thimet granules. 1957.

Thimet per Tree (Oz.)	Leaf Miners		Per Cent Kill
	Dead	Alive	
	Treated July 18[a]		
0.08	90	4	95.7
.16	94	6	94.0
.24	113	0	100.0
.32	168	0	100.0
Untreated	7[b]	83	
	Treated August 9[c]		
0.005	40	30	57.1
.01	22	5	81.4
Untreated	0	43	

[a] Results recorded October 7.
[b] One parasitized.
[c] Results recorded October 1.

growing in nursery rows. Thirty-two plants received the treatment at the rate of 2 oz. of 2% granules and 22 plants at the rate of 1 oz. per plant. The material was raked into the soil but no watering was done.

A second experiment was undertaken on July 19 using 6- to 8-foot holly trees. In this instance Thimet was sifted onto the soil under the trees as far as their periphery. A 2% granulated formulation was used at the rate of 4, 8, 12 or 16 oz. per tree. Treatments were not replicated. They were thoroughly drenched with water.

Data on control were taken from both series of experiments between September 25 and 30. The mines in 40 of 80 leaves taken at random from each treatment and from the controls were dissected for results of the tests which are presented in table 2.

Table 2 shows the effectiveness of small amounts of Thimet in controlling holly leaf miners. Furthermore, it may be noted that a dose of 4 oz. of 2% Thimet gave good control of this leaf miner in a 6- to 8-foot tree. The treatments did not cause any injury to the plants.

CONTROL OF BOXWOOD LEAF MINER.—On July 18 granules containing 2% of thimet were sifted onto the soil under four 3-foot boxwood plants having circumferences of 5 to 6 feet. The treatments were made at the rate 0.08, 0.16, 0.24 or 0.32 oz. of actual Thimet per plant. Water was used to soak the treatments into the soil at the time of application and thereafter at about weekly intervals until early September.

An additional experiment was undertaken on August 9 using the 2% granules on the soil under two boxwood hedges. The hedges were 12″ to 18″ high, 12″ wide at the top and 8′ long. The systemic was used at the rate of 4.75 or 9.5 oz. of a 2% formulation per 100 sq. ft. of ground. The concentrations applied 40 and 80 ounces, respectively, of Thimet per acre or 0.005 or 0.01 oz. per plant (planted at 3′×3′ intervals).

Counts of this leaf miner made on the basis of 10 leaves per sample, taken at random from the hedges prior to treatment, indicated an average of 4.1 larvae per leaf were present.

Data on control were taken on October 1 and 7 and are given in table 3. The mines in 20 leaves from each treatment and the check were dissected for determining the results. These leaves were taken at random from 20 twigs (averaging 1 to 5 inches in length) cut from each lot.

The data given in table 3 show the effectiveness of Thimet soil treatments in controlling boxwood leaf miner. Obviously, the lower doses (comprising the two boxwood hedges treated August 9) were less efficient in destroying leaf miners than when the applications were made to individual plants at higher levels on July 18. At the rate of 4500 ± plants per acre (spaced 3′×3′ apart), a dosage of 80 ounces of the active ingredient of Thimet per acre would achieve a 0.01-oz. level per plant. This was actually only one-eighth of the lowest concentration employed when the plants were treated individually. Thimet soil treatments did not appear to injure boxwood.

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NOTICE TO AUTHORS

Authors are urged to consult the Publication Policies and Manuscript Rules and Suggestions (Vol. 49(4): 575–7) before preparing manuscripts intended for publication in the JOURNAL OF ECONOMIC ENTOMOLOGY.

SCIENTIFIC NOTES

Japanese Beetles: Differences in Damage to Varieties of Sweet Corn and Control with DDT

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Japanese beetles were in greater abundance during the sweet corn silking period of 1957 than had ever been observed at Faison, N. C. in previous seasons. Adults began to appear in May and reached their population peak during late May and early June. Langford *et al.* (1944) in Maryland, found that early planted (May 13) field corn attacked by Japanese beetles suffered a loss in grain of from 14.4 to 39.6%. Late planted (May 31) corn suffered a loss of from 4.6 to 26.3%. They observed the amount of damage varied in varieties but that when silking occurred after August 10 the loss was not above 9.0% for any variety. However, late planted sweet corn may be severely damaged by fall armyworm in North Carolina (Brett 1953).

Sweet corn varieties, Ioana (Asgrow), Goldenyield (Michael-Leonard), and Calumet (Asgrow) were planted in plots four rows wide and 50 feet long. Each treatment and check was replicated four times. A number of other varieties were tested but variation in silking time made a comparison of results rather difficult.

Sprays were applied 2 days after the first silks appeared, and at 4-day intervals thereafter for a total of three applications. The spray formulation contained 5 gallons of 25% emulsifiable DDT in 100 gallons of water. This was used at the rate of 25 gallons per acre. A 4-gallon garden tank sprayer was used and sprays were directed into the silks. About 2 weeks after the first appearance of silks, the number of Japanese beetles on 25 silks was counted in each plot. Totals (100 silks from four plots) were as follows: Ioana had four beetles on the treated silks and seven on untreated ones. Calumet had no beetles on the treated silks and 14 on the untreated ones. Goldenyield had one beetle on the treated silks and 208 on the untreated ones. The untreated Goldenyield silks were almost entirely destroyed and considerable pollination injury was apparent in the reduction of kernel development. This did not occur in any of the other plots.

This experiment shows very good control of Japanese beetles in sweet corn silks with DDT sprays. It also appeared that some sweet corn varieties had silks which were more attractive to Japanese beetles than other varieties, under the conditions of this experiment. Testing of variation in damage to sweet corn varieties by Japanese beetles will be continued.

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Moranila californica as a Usurped Parasite of *Saissetia oleae*[1]

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The hymenopterous parasite, *Moranila californica* (How.), is extremely scarce in California, yet, at the beginning of this century it was more abundant as a parasite of the black scale, *Saissetia oleae* (Bern.), than *Scutellista cyanea* Mots. is today.

Comstock (1881) noted that in 1880 enormous quantities of the eggs of black scale were destroyed by *Moranila* (= *Tomocera*). Ehrhorn (1901) reported to the 26th California Fruit Growers Convention held in San Francisco in December, 1901, that *Moranila* reduces black scale infestations from 30 to 75%. He stated, however, that the remaining scales contain sufficient eggs to again infest the host plant. Ten years later Quayle (1911) found that *Moranila* had become so scarce that he could not obtain "enough material to make it worth while undertaking life history studies."

Quayle found it difficult to account for the change from abundance to scarcity. He suggested, however, that the usurping of the field by *Scutellista* subsequent to its introduction in 1902 as the definite and specific cause. This suggestion seems confirmed by a similar change in status of *Moranila* in Hawaii, where *Scutellista* was introduced in 1905 (Smith & Compere 1928). These authors found that *Moranila* is readily propagated on black scale in the absence of *Scutellista*. It was observed that under natural conditions *Moranila* usually deposited an egg whenever its ovipositor was inserted under a gravid female scale.

The life histories of *Moranila* and *Scutellista* appear to be quite similar. The time required for *Moranila* to deposit an egg in the brood chamber of the black scale varies from ½ to 2 minutes. An egg may leave the ovipositor 7 seconds after its insertion beneath the scale.

At a constant temperature of 82° F. the life cycle is 29 days, the egg incubation period 3 days, the 1st larval stage 4 days, the 2nd larval stage 2 days, the 3rd larval stage 8 days, and the pupal stage 12 days. The newly hatched larva has four pairs of spiracles and is smaller than the black scale egg. It is able to grow for a short time in the absence of scale eggs by feeding on the body fluids of the mother scale. The larva differs from that of *Scutellista* in possessing six rows of hair-like spines on the body.

Moranila is bisexual, but under insectary propagation it reproduces uniparentally (Flanders 1952).

The species now appears to be world wide in its distribution, having been collected in Africa and Australia, as well as in Hawaii and California.

Prior to 1902 when the introduced *Scutellista* was released, *Moranila*, as a parasite of black scale in California, had no parasitic competitors of any importance. *Coccophagus scutellaris* (Dalman) and *Metaphycus luteolus* Timb. were relatively scarce as parasites of black scale (Smith & Compere 1928).

The prompt replacement of *Moranila* by *Scutellista* can be accounted for by the probability that *Moranila* is alien to black scale. This is indicated by a difference in their oviposition habits. The black scale is characterized by a posterior arch through which the newly hatched larvae, escape from the brood chamber. *Scutellista*, in preparing to oviposit, searches for this arch and utilizes it for the insertion of the ovipositor. *Moranila*, however, probes at any point around the periphery of the scale making many futile attempts before finding a point of insertion.

The effect of the importation and establishment of the African parasites, *Metaphycus lounsburyi* How., *M. helvolus* Comp., and *M. stanleyi* (Comp.), on the status of *Moranila* is unknown. Competition may, in time, result in the elimination of *Moranila californica* from the California scene.

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Feeding of Four Species of Fabric Pests on Natural and Synthetic Textiles[1]

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Tests were conducted with four species of fabric pests to determine if they feed on synthetic fibers and on combinations of wool and synthetic fibers. The larvae of the webbing clothes moth (*Tineola bisselliella* (Hum.)), the black carpet beetle (*Attagenus piceus* (Oliv.)), the furniture carpet beetle (*Anthrenus flavipes* Lec.) and the adults of the firebrat (*Thermobia domestica* (Pack.)) were exposed under starvation conditions to the following synthetic fibers: nylon, Dynel, Dacron, Orlon, Vicara, acetate rayon, and viscose rayon. Natural fabrics of silk, wool, linen, and cotton were also subjected to attack by these insects, as were combinations of wool and nylon, wool and Orlon, and wool and rayon.

Lindsay (1939) noted that artificial silk and cotton were eaten by *Ctenolepisma longicaudata* Esch., and that wools and silks were not eaten. Slabaugh (1939) reported that *Thermobia domestica* (Pack.) eats rayon, preferring the finishing agents such as starch and gums to the rayon itself. Mallis (1941) conducted tests with *Ctenolepisma urbana* Sla. under natural conditions, and found that this species fed on linen, rayon, and cotton. Linen was the preferred fiber. The feeding on natural silk and wool was negligible. Austin & Richardson (1941) found that regenerated cellulose, either knitted or plain weave, and linen were the materials most heavily damaged by the firebrat; cotton and silk were attacked if the texture was suitable for feeding. Patton (1945) showed that several species of carpet beetles, especially the varied carpet beetle (*Anthrenus verbasci* (L.)), made holes in nylon. Moncrieff (1950) noted that mixtures of wool and nylon and wool and rayon may be attacked by clothes moths. He also states that carpet beetles eat all animal fibers, including silk, as well as the glue, casein and albumin sometimes used as sizes on rayon and other fabrics made from cellulose. Wall (1953) found that *Thermobia domestica* (Pack.) and *Ctenolepisma quadriseriata* (Pack.) fed on viscose rayon and wool. It may be seen from the above references to the literature that insects feed on several synthetic fabrics. Because of a dearth of published information on the feeding by insects on the newer synthetic textiles such as Orlon, Dacron, Dynel, Vicara, and mixtures of these with wool, tests were undertaken with these and other textiles.

PROCEDURE.—Experiments were conducted with the webbing clothes moth, the black carpet beetle and the furniture carpet beetle with slight modifications of the CSMA Textile Resistance Test (Anon., 1952). Circular pieces of test cloth, 2 sq. inches in area, were placed in 3-oz. seamless tin boxes, $2\frac{3}{4}''$ diameter by 1" high with a 60×60 mesh screen 2" in diameter which was soldered in the lid. Four replicates were conducted with each test fabric. Ten clothes moth larvae, 24 days old from the date of egg deposition, were placed in each test container. Larvae of the black carpet beetle, used in the tests, passed through a U. S. Standard Sieve Series No. 14, and were retained on a No. 16 Sieve. The larvae of the furniture carpet beetle used measured from 2 to 3 mm. in length. The carpet beetle and clothes moth larvae were reared according to specifications in the Chemical Specialties Manufacturers Association (CSMA) Textile Resistance Test. The textiles were exposed to the larvae for 2 weeks in a room with a temperature of 82° F.±2° and in average relative humidity of 40%. Ten larvae were placed on a sample, and four replicates were run with each sample.

The procedure for testing the feeding of the firebrats differed slightly from that for the clothes moths and carpet beetles. A rectangle of the test fabric, 1"×2", was placed in a 2-oz. straight-sided wide-mouth (French square) bottle. Ten firebrats, 7 to 10 mm. in length, were placed on each test cloth. The firebrats were reared in a constant temperature chamber with an average temperature of 90° F. and a relative humidity of 40%. The test containers were maintained throughout the test in the above chamber except when they were removed for observation. The firebrats were exposed to the test cloth for 4 weeks. Two replicates were conducted with each sample.

The damage to fabrics was determined by visual examination, except where very slight damage was confirmed with a binocular microscope. The feeding on the test fabrics was rated as follows:

- O No damage
- VS Very slight damage confirmed by examination under a binocular microscope (21.25 magnification)
- S Slight feeding along edges and/or slight feeding on nap
- E Extensive feeding along edges and/or holes through cloth

RESULTS.—Table 1 shows the amount of feeding on test fabrics by the webbing clothes moth, the black carpet beetle, the furniture carpet beetle, and the firebrat.

Table 1.—Feeding on fabrics by four species of insects.

TEST FABRIC	AMOUNT OF FEEDING ON TEST CLOTH[a]			
	Clothes Moth	Black Carpet Beetle	Furniture Carpet Beetle	Firebrat
Synthetic fabrics:				
Nylon	O	S	S	O
Dynel	O	O	O	O
Dacron	O	VS	O	O
Orlon	O	O	O	O
Vicara	O	VS	VS	O
Acetate rayon	VS	O	E	VS
Viscose rayon	O	VS	O	E
Natural fabrics:				
Silk crepe	O	S	VS	S
Wool (AATCS)	E	E	E	O
Linen	VS	O	O	S
Cotton percale	VS	O	VS	VS
Combination of wool and synthetic fabrics:				
86% wool and 14% nylon	E	E	E	O
45% wool and 55% orlon	E	E	E	O
45% wool and 55% viscose rayon	E	E	E	VS

[a] Rated as follows: O, no damage; VS, very slight damage; S, slight feeding; E, extensive feeding.

In interpreting the test results, the category "VS" (very slight damage) can be considered as "O" (no damage) because the feeding was of an exploratory nature on individual fibers and resulted in no real damage, and is probably of no practical significance. We can see from the above results that the clothes moth did no damage to the synthetic fibers, but definitely attacked the combination of wool and synthetic fibers.

Both the black carpet beetle and the furniture carpet beetle extensively damaged combinations of wool and synthetic fibers. Both species also caused slight damage to nylon. Whereas, the black carpet beetle did not feed on acetate rayon, the furniture carpet beetle fed extensively on the acetate rayon fabric.

The firebrat caused extensive damage to viscose rayon and slight damage to linen and silk; it did not feed on the other fibers.

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Oil-Base Sprays against Larvae of the Black Carpet Beetle[1]

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Twelve oil-base household sprays were tested in the laboratory against larvae of the black carpet beetle, *Attagenus piceus* (Oliv.). In general, the concentration of insecticide used in the spray was the amount recommended for household use by the manufacturers. The speed of action and effectiveness of the sprays were observed over a period of 28 days.

The insecticides were dissolved in Gulfspray Naphtha (a typical odorless type household insecticide base oil, 0.78 sp. gr., and 380 to 480° F. distillation range). In order to get the required amounts of DDT and methoxychlor in solution, it was necessary to use an auxiliary solvent, 15.9% by weight of Solvent 2A, an aromatic petroleum fraction.

The test insecticides were applied as direct sprays by a procedure used against cockroaches and other insects (Miller *et al.* 1954). This test is believed to approximate the application of sprays in the house, and employs a stainless steel hoop and a modified Peet-Grady atomizer.

The black carpet beetle larvae used in the test were raised according to specifications of the Chemical Specialties Manufacturers Association (CSMA) Textile Resistance Test (Anonymous 1952). The larvae passed through a U. S. Standard Sieve No. 14, and were retained on a No. 16 Sieve.

Twenty larvae were used in each test, and nine tests were run with each insecticide. The larvae were placed in the center of the steel hoop, which is 32 inches in diameter, and were permitted to move freely. The steel hoop rested on grey bogus paper, which was renewed after each application of spray.

A dosage of 6 ml. of the test insecticide at a pressure of 12½ p.s.i. gauge was applied with the modified Peet-Grady atomizer. The nozzle of the atomizer was held about 20 inches from the paper, and the gun was moved in a circular fashion so that the entire area within the hoop was sprayed. The object was to distribute the spray evenly over the area enclosed by the hoop without making any special effort to wet the larvae. All parts of the paper within the hoop were slightly wet by the spray.

The larvae were exposed in the hoops for 10 minutes and then picked up with forceps and placed in clean crystallizing dishes. These were kept in a room with a constant temperature of 82° F. ± 2° and a relative humidity of 40%. The larvae were not provided with food because previous tests have shown that they can survive for months without food. Observations were made at intervals of 1, 2, 3, 6, 14, and 28 days, and the number of alive, dead, and moribund larvae were noted. A normal larva is usually quiescent and lays upon the bottom of the glass dish with the dorsal surface uppermost. A moribund larva usually lays upon its side. It may curl its body, twitch, shorten in length, and eventually darken in color and dry up. Because the larvae may "play possum," it was necessary to gently probe and carefully examine them for voluntary movements under a binocular microscope with a magnification of 21.25 diameters. A dead larva was one that showed no voluntary movement when gently probed.

Table 1 shows the insecticides and concentrations used, and the average per cent moribund or dead larvae (nine replicates

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Table 1.—Effect of oil-base sprays against larvae of the black carpet beetle.

Insecticide (Per Cent by Weight)	Average Per Cent Moribund and Dead												Average Per Cent Moribund and Dead 28 Days
	1 Day		2 Days		3 Days		6 Days		14 Days		28 Days		
	M	D	M	D	M	D	M	D	M	D	M	D	
6.0 DDT[a]	99	1	99	1	99	1	90	9	12	87	1	98	99
6.0 methoxychlor[a]	99	0	90	1	88	3	74	11	37	35	32	47	79
6.0 Perthane	95	0	90	1	88	3	73	12	38	34	12	47	59
2.0 chlordane	49	0	52	3	51	4	66	8	41	26	27	40	67
0.5 dieldrin	61	0	59	0	45	2	29	5	23	10	10	17	27
0.5 lindane	95	1	94	1	91	2	57	8	20	29	2	42	44
2.5 Strobane	39	2	40	2	39	4	26	7	15	17	6	26	32
3.0 malathion	100	0	99	1	99	1	74	26	55	45	42	58	100
0.5 Diazinon	98	1	93	1	83	4	84	9	59	31	26	60	86
0.136 pyrethrins[b]	84	1	27	2	17	3	6	11	1	19	1	20	21
0.128 pyrethrins+ 0.128 piperonyl butoxide	96	0	54	1	18	4	7	11	2	16	1	18	19
8.0 Lethane 384 Special[c]	1	0	2	0	1	1	0	2	1	2	1	2	3
100.0 Gulfspray Naphtha	14	1	12	2	9	4	4	10	1	14	0	17	17
15.9 Solvent 2A	8	1	7	1	7	3	2	8	1	11	0	13	13
Untreated check	1	0	1	1	1	1	0	1	0	1	0	1	1

[a] 15.9% by weight Solvent 2A, an aromatic distillate used as an auxiliary solvent.
[b] Official Test Insecticide of Chemical Specialties Manufacturers Association—approximately 106 mg. pyrethrins/100 ml.
[c] Lethane 384 Special consists of 12.5% by volume 2-butoxy-2′-thiocyanodiethyl ether and 37.5% by volume beta-thiocyanoethyl laurate standardized with petroleum distillate.

were used for each insecticide tested, or a total of 180 larvae for each test).

The results presented indicate that DDT, methoxychlor, Perthane, lindane, malathion, Diazinon, pyrethrins, and pyrethrins plus piperonyl butoxide affected the larvae most rapidly. DDT, malathion, Diazinon, and methoxychlor showed the greatest average per cent moribund and dead at the end of 28 days. Practically no larvae were normal 28 days after being sprayed with either DDT or malathion.

Larvae unaffected by the sprays at the end of 28 days were exposed to fresh woolen test cloth to determine if they were still capable of feeding. Because practically no larvae survived treatment with DDT or malathion, feeding tests were not conducted with larvae sprayed with these insecticides. Normal larvae that survived treatment with chlordane for the most part did not feed on untreated test cloth. All other normal larvae that survived the spraying fed on untreated test cloth 28 days after being sprayed with the test insecticide.

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The Use of Vermiculite in Medium for Stable Fly Larvae[1]

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Although the stable fly (*Stomoxys calcitrans* (L.)) is being reared successfully in several laboratories, it is generally believed to be more critical in its requirements than the house fly (*Musca domestica* L.). One of the requirements for rearing the stable fly is a good medium for the larvae. Campau *et al.* (1953) and Champlin *et al.* (1954), used the standard Chemical Specialties Manufacturers Association (CSMA)[2] medium developed for house fly rearing. McGregor & Dreiss (1955) diluted the medium with wood shavings but did not add malt or yeast. Better results were obtained in this laboratory with the use of the standard CSMA medium in low, round bottom 1-gallon crocks than in the deeper cylindrical type. Recently it was found that improved results were obtained by the addition of vermiculite to the standard medium.

The proportions and directions for mixing the medium are as follows:

Part I	
Water	3892 ml.
Yeast suspension (20%)	232 ml.
Diamalt	32 ml.
Fly medium (Ralston-Purina)	1828 gm.
Part II	
Vermiculite (Terra-Lite)	400 gm.
Water	2000 ml.

Part I is prepared by mixing the water, yeast and Diamalt and combining the mixture with the fly medium. Part II is made by adding water to the vermiculite. Part I and Part II are stirred together to make a blend of small aggregates of fly medium and damp vermiculite. This amount is sufficient for five flat 1-gallon earthenware crocks. Each crock is seeded immediately with 0.6 ml. of washed eggs. The crock is covered with muslin, held in place with a rubber band, to prevent stray flies from depositing eggs and placed in the fly room at about 80° F. for 12 days. At this time nearly all the larvae will have pupated and can be separated. Some will start emerging on the 14th day. The pupae are cleaned by the air separator described by Goodhue & Linnard (1950).

The reason for the improved results with vermiculite has not been determined with certainty. The vermiculite may act as a diluent which reduces the rate of fermentation and prevents a rapid rise in temperature. It may be an adsorbant for the fermentation products or the regions high in vermiculite may be a place of refuge for the larvae.

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[1] Accepted for publication December 12, 1957.

[2] Purchased from Ralston-Purina Company, St. Louis, Missouri.

Control of *Nysius raphanus* Howard Attacking Vegetables[1]

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Nysius raphanus Howard, one of the so-called false chinch bugs, was reported for the first time feeding on lettuce, cabbage, turnips, and mustard greens in the Lower Rio Grande Valley during February 1952 (Wene 1953). The insect reappeared in damaging numbers during February 1954 and 1956. Both of these destructive infestations occurred on vegetables during periods of prolonged drought. The vegetation in the surrounding uncultivated fields was dying due to a lack of soil moisture and the bugs had migrated to the more succulent vegetation in the irrigated fields. Apparently these were definite migrations since all those observed developed to destructive numbers within a week or less.

Severe infestations of this insect destroyed some lettuce fields in 4 or 5 days. About 70 individuals per square inch were noted in some fields. The initial feeding of the insect was restricted to the dorsal surface of the outer leaves of the lettuce plant. In a day or so the infested leaves collapsed and fell to the ground, after which the bugs started feeding on the lettuce heads which soon became grayish white in appearance. On turnips the insects were noted feeding largely on the ventral surface of the leaf in a more or less gregarious manner. This resulted in killing the area of the leaf under attack, after which the bugs moved to another area on the turnip leaf. The insect also occurred in large numbers on cabbage plants but no apparent damage was observed; however, cabbage harvested from an infested field was rejected by vegetable shippers because of the presence of the insects on the outer leaves.

Previous work (Wene 1953) indicated that *Nysius raphanus* could be controlled with aldrin or with a combination of BHC and DDT. Toxaphene was not so effective as aldrin whereas parathion failed to give any control. Tests on the effectiveness of various insecticides in controlling this insect were repeated in 1954 and 1956.

Procedure.—The insecticidal dusts were applied with rotary hand dusters at a rate of 25 pounds per acre. Experiment 1 was conducted in a turnip field and experiment 2 was conducted in a

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lettuce field. Each treated plot was 0.02 acre in size, and each treatment was replicated three times (table 1).

The spray treatments were applied as low volume sprays. A 3-gallon garden sprayer was constructed so that the sprays were applied at the rate of 5 gallons per acre. Each treated plot consisted of a single row of lettuce 30 feet in length. The rows were spaced 40 inches apart and an untreated row bordered each treated plot.

Table 1.—Control of *Nysius raphanus* with various insecticides at Weslaco, Texas.

Insecticide[a]	Number of Bugs per Leaf on Days After Treatment Shown		
	1	3–4	6–7
Experiment 1 (Dusts)			
Heptachlor 5%	2.2	1.4	3.6
Aldrin 2.5%	1.6	1.5	5.0
Endrin 2%	0.5	0.4	1.8
Toxaphene 20%	1.5	2.4	3.0
Untreated	9.3	9.4	16.3
Experiment 2 (Dusts)			
Sulphur	22.3	15.3	—
Piperonyl Butoxides 0.9% +Pyrethrins 0.06%	15.0	12.0	—
Pyrethrins 0.1% (Impregnated)	4.3	2.5	—
Malathion 5%	1.2	2.1	—
Untreated	40.0	27.0	—
Experiment 3 (Low Volume Sprays)			
Dieldrin 0.38	3.1	2.0	1.8
Malathion 1.25	1.7	0.2	1.2
Schraden 1	3.6	1.1	1.2
Demeton 0.25	1.2	1.4	1.8
Phosdrin 1	0.3	0.1	1.8
Untreated	30.2	34.3	22.2
Experiment 4 (Low Volume Sprays)			
Malathion 1.25	1.5	1.8	—
Malathion 0.6	2.8	3.6	—
Phosdrin 0.5	0.3	0.4	—
Phosdrin 0.25	0.0	0.2	—
Diazinon 0.25	4.4	2.0	—
Chlorthion 0.25	1.9	1.9	—
Methyl parathion 0.25	1.4	1.5	—
Untreated	16.1	21.0	—

[a] Dusts were applied at 25 pounds per acre. The low-volume sprays were applied at 5 gallons per acre containing the number of pounds of toxicant shown in this column.

Effectiveness of the various insecticides was determined by selecting 10 leaves at random in each plot and counting the surviving insects at various time intervals after the applications were made.

Discussion of results.—The data in table 1 show that dust formulations of heptachlor, aldrin, toxaphene, and endrin resulted in effective control of this insect. Endrin remained effective for a 7-day period whereas the other insecticides started to lose their effectiveness 7 days after the applications. In experiment 2, as shown in table 1, dusting sulphur and a mixture of 0.9% piperonyl butoxide with 0.06% pyrethrins failed to give adequate control. Good control of the bug was obtained with 5% malathion or 0.1% impregnated pyrethrum dust.

The data obtained in experiments 3 and 4 indicate that this bug can be controlled with the following insecticides applied as low volume sprays: 0.38 pound dieldrin; 1 pound schraden; 0.25 pound concentrations of methyl parathion, Phosdrin, Diazinon, demeton and Chlorthion; and 1.25 pounds of malathion per acre. Malathion at 0.6 pound per acre was not so effective as at the higher dosage.

Malathion proved effective in contolling this bug but in the malathion-treated plots the dead insects adhered tightly to the leaf surface and were difficult to shake or wash off and this proved very objectionable to shippers. The adherence of dead insects to the host crop was not observed with any of the other insecticides tested.

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Life History and Control of a Root Collar Borer (*Euzophera ostricolorella* Hulst) in Yellow-Poplar[1]

C. John Hay[2]

The larvae of a Lepidopterous borer, *Euzophera ostricolorella* Hulst, reported in the eastern United States from New York to Georgia, have been found boring in the inner bark of yellow-poplar (*Liriodendron tulipifera* L.). Although it will probably be found throughout the range of its host, heretofore apparently this borer has not been considered economically important.

Hulst (1890) originally described the adult. Other descriptions by Forbes (1923) and Heinrich (1956) followed. Heinrich (1921) described the larva and pupa.

Detection and Progress of Infestation.—An infestation was reported in western Kentucky and Tennessee October 1954. At that time yellow-poplar trees of all sizes, but chiefly those larger than 10 inches d.b.h., were observed dead or dying on a 5,500-acre timber tract on rich limestone land lying along the Cumberland River just below Lamasco, Kentucky, in Lyon and Trigg Counties. There were also signs of borer work in some poplars in western Tennessee although no dying trees were detected. The borer has been observed in eastern Kentucky, but only rarely.

The accelerated infestation has probably been in progress for 4 or more years. Just what contributed to the buildup in the Lamasco area is not known. There has been no severe drought in the area and infested trees have made good to excellent growth during the past 5 to 7 years. Observations made in and around the Lamasco tested area indicate that the population has been decreasing for the last 2 years. In the fall of 1956 few borers were found, and recently attacked trees were scarce. Usually not more than one live larva or pupa was found per attacked tree.

Notes on Life History.—The main flight of adult moths apparently comes early in the spring. During 1955 there was evidence of a partial fall emergence, thus suggesting that one and a partial second generation may occur in 1 year. Larvae arising from the egg laying of this fall emergence were found to be approximately one-half inch in length in the middle of April, 1956. The fully mature larva is about 1 inch long.

Larval development is completed late in the summer or early fall and a cocoon is spun in the burrow in which the larva pupates and overwinters. Pupation takes place with the head facing the nearby exit hole. When the adult emerges, the empty pupal case is left within the cocoon which remains in place in the larval tunnel.

Description of the Attacks.—Although few in number, attacks were found on trees as small as 6 inches in diameter breast height. The rate of attack increased greatly in trees over 10 inches. More than 90% of the attacks were within 2 feet of the ground. A few were found as high as 10 feet but these generally appeared to be unsuccessful. The attacks were concentrated in an area extending from 2 inches below the duff to 5 inches above, thus indicating the preference of the insect for a moist

[1] Accepted for publication January 13, 1958.

[2] Central States Forest Experiment Station, U. S. Forest Service Columbus Ohio.

habitat. The burrows were extended more or less vertically by the larvae above or below the duff line. Tunnels were seldom more than 4 inches in length and about one-fourth inch in diameter. Most of the burrows were confined to the soft phleom tissue. Occasionally a larva penetrated to the softwood, but did not etch it. The chamber walls and surrounding wood were stained with a deep black color and a blackish ooze accompanied by frass flowed from the entrance hole, staining the bark surface for a few inches below the hole. Small larvae were often found burrowing adjacent to old injuries with their tunnels originating in the old tunnel cavities. The coarse frass was therefore pushed into the old tunnels and could not be detected on the bark exterior.

EFFECT OF SALVAGING.—Early in the summer of 1955 a salvage cut was made in the Lamasco area, where all heavily attacked dead or dying trees were removed. The following spring the stumps of these trees were examined for brood survival. Mature larvae and pupae and evidence of fall emergents were detected in the stumps. The small larvae such as were present in living trees at this time could not be found in the stumps. This is an indication that stumps are undesirable as sites for egg deposition. Since broods that were in the stumps at the time of cutting were able to complete development, the borer population was not greatly reduced by the salvage operation.

LUMBER DEFECT.—Typical callous wound tissue was seen to form over old attacks; this was discernible especially where attacks occurred above 5 inches on the bole. A small defect is left in the wood. However, because so few attacks occur above stump height, degrade is not a serious problem. Moreover, defect in the logs can be slabbed off or peeled away in veneer with a resultant small loss of footage.

NATURAL ENEMIES.—Although no woodpeckers were observed in the area, their presence was attested by their work on nearly every borer-infested yellow-poplar. No parasites were present in host material that was reared in the laboratory. However, in several instances in the field empty Hymenopterous or Dipterous pupal cases were found in the borer tunnels.

REMEDIAL AND PREVENTIVE CONTROL TESTS.—In April 1955, fifteen yellow-poplar trees 8 to 40 inches d.b.h. were treated with a water emulsified spray containing 0.5% of the gamma isomer of BHC and 2% DDT. Fifteen trees were held as checks. The trees were scattered over a test area of about 20 acres of bottomland hardwoods near Lamasco, Kentucky. Some of the trees bore only a few attacks; others were attacked all around the base. Treated and non-treated trees were interspersed throughout the test area.

The spray was applied with a compressed-air garden sprayer to the basal 6 feet of the trunk. Two-thirds of a gallon was the average amount applied per tree. The applications were made just prior to the anticipated adult-emergence period.

The emulsion treatment did not prevent attacks: about 50% of the attacking brood survived. (Table 1). The check trees showed about twice as many attacks, but again survival was 50%. So a second treatment was made the following spring. The same ingredients were used, but this time in an oil solution. No new spring attacks could be detected following the application, thus indicating successful preventive control. It also appears that remedial control was obtained because only dead larvae were found the following fall. These larvae originated from attacks in the previous fall, prior to the spring treatment.

Only two trees died. Both were check trees of poor vigor and both had been riddled with prior attacks around the base. Although the sudden decrease in borer population in the infested area made it impossible to precisely analyze the results, it would appear that the oil solution is superior to the emulsion in controlling this insect.

Table 1.—Effectiveness of treatments with 0.5% BHC plus 2% DDT for control of root collar borer in yellow-poplar.

		Number of			
		Attacks by Young Larvae Prior to Treatment	Attacks Following Treatment	Brood Survival	
Formulation	Trees			Number	Per Cent
Water Emulsion	15	0	12	7	58
Check	15	0	30	14	47
Oil Solution	18	20	0	0	0
Check	15	15	5	1	5

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Collembola Damage to Seedling Cotton[1]

B. L. OWEN[2] and W. L. OWEN, JR.[3]

A species of Collembola, *Entomobrya unostrigata* Stach., damaged seedling cotton over a wide area of the southern High Plains of Texas and eastern New Mexico during the years, 1955 through 1957. Similar damage to cotton had not been previously observed. Injury to plants occurred from about May 10 to June 15, depending on planting date. The area affected would normally produce 1½ to 2 million bales of cotton annually.

The injury (fig. 1) was characterized by few to numerous holes in the cotyledons of most plants and partial to complete destruction of the cotyledonous leaves of many plants. The base of the cotyledons was often damaged while they were still almost completely covered by the seed coat. This occurred in the crook stage before the plants emerged. Lesser damage occurred after plants appeared above ground. In both cases, injury first appeared as rasped areas, reaching only part way through the cotyledons, the remaining tissue of the rasped areas dried and fell away leaving the characteristic holes.

Numerous observations showed that heaviest damage was restricted to fields which had large amounts of plant residue in the soil. Seedling damage was greatest in fields where cotton gin trash had been added to the soil the previous fall; however, damage occurred in fields having large amounts of plant residue from previous crops, primarily grain sorghum and alfalfa.

Tremendous numbers of this species of Collembola and noticeable numbers of Coleoptera belonging to the families Anthicidae and Staphylinidae, were always observed where plant damage occurred. The beetles and Collembola were in the surface litter and in the soil of these fields. The insects, when found in the soil, were usually immediately around or on plants which had not emerged.

Although the large populations of Collembola seemed to indi-

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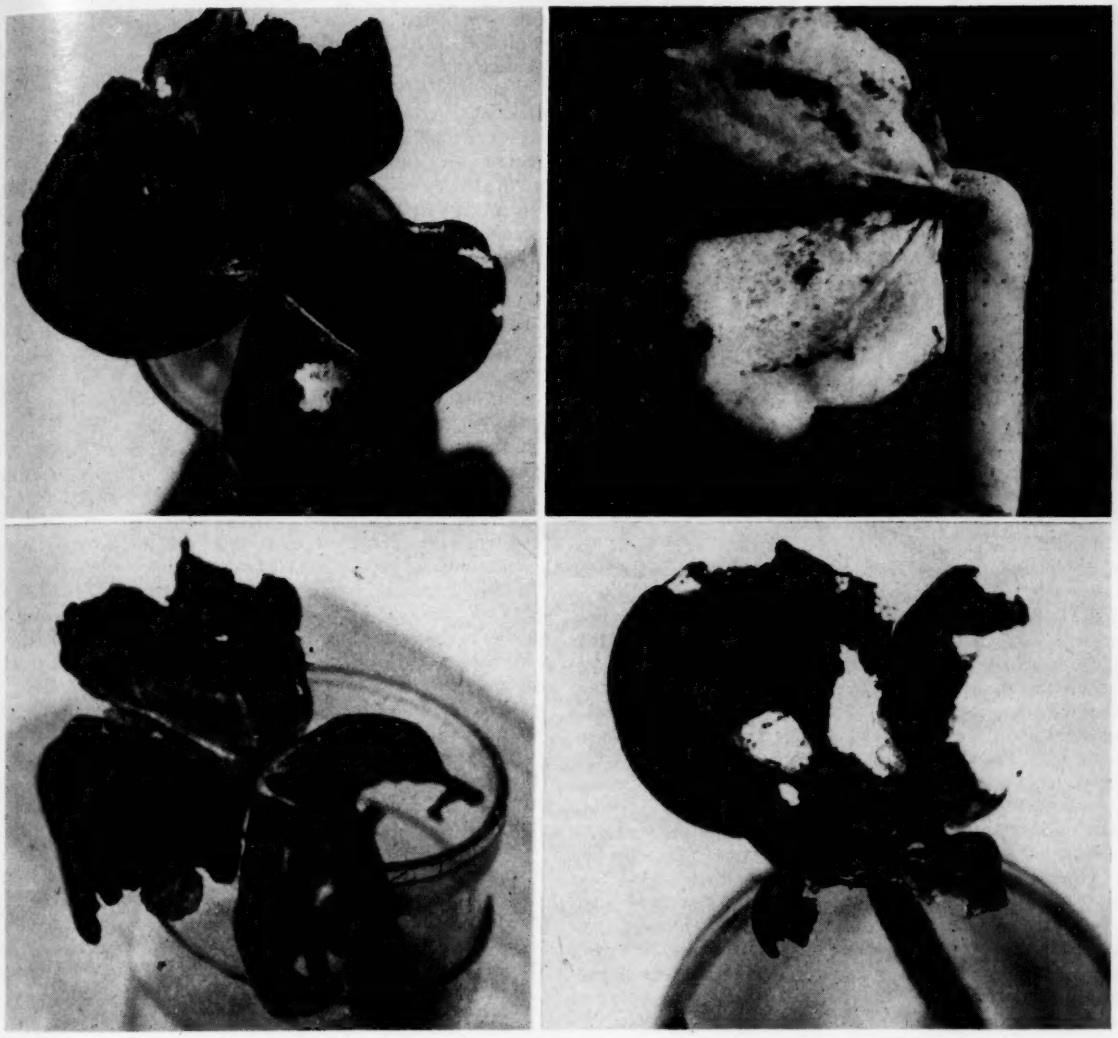

FIG. 1.—Collembola damage to seedling cotton.

cate these insects were causing the plant damage, laboratory studies were conducted in 1955 for positive determination. Cottonseed were planted in several types of soil, including sterilized soil from fields where cotton had suffered heaviest damage. A number of insects, separated as to species, were placed in cages on the soil at various stages of seed germination and plant emergence. No appreciable typical damage was caused by either the anthicids or staphylinids. Light to heavy damage, depending upon the population, was caused by the Collembola. This damage was the same as that observed in cotton fields.

Damage to cotton was much the same during each of the 3 years; however, complaints from farmers were much more general during 1956 and 1957. During all 3 years, where seeding rates were ample (1 bushel per acre), stands were not reduced to a serious degree. With lower seeding rates, heavy infestations of the insects left poor stands. Replanting was necessary in a number of fields following serious damage. Populations of the insect and damage were much heavier where cotton gin trash had been applied to the soil the previous fall. In all 3 years, populations in the surface trash and in the upper soil layer decreased as soil dried out.

To further indicate the recent appearance of these insects in large numbers on the High Plains, they were first observed in lawns in midsummer of 1956. It was also the first year they invaded homes in sufficient numbers to cause widespread complaint. During the early summer of 1957 they were first observed in large numbers in small grain fields.

The Collembola were identified by Dr. K. A. Christiansen[4] as *Entomobrya unostrigata* Stach. Christiansen (1956) reported that the species was collected in Colorado in 1950, in California and Utah in 1952. He stated that since the form is so striking in the type of pattern and mucronal form, it seems very unlikely that any of the major investigators would have encountered it at an earlier date without recording its presence. In view of these facts and the abundance of the insects where they are found, Christiansen stated that it is likely that this form has recently been introduced into North America.

The species is easy to recognize by two structures: the minute but definite anteapical mucronal tooth, and (in patterned forms) the existence of a median dorsal line (Christiansen 1956).

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Control of Three Species of Cabbage Caterpillars with Some New Insecticide Dusts[1]

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One of the major cabbage growing areas in North Carolina is in the mountains near Fletcher. Considerable difficulty is reported here from time to time in controlling cabbage caterpillars. Species usually present are the cabbage looper (*Trichoplusia ni* (Hbn.)), the imported cabbageworm (*Pieris rapae* (L.)), the cross-striped cabbageworm (*Evergestis rimosalis* (Guen.)), and the diamondback moth (*Plutella maculipennis* (Curt.)). These are listed in about their order of importance and frequency of occurrence. Toxaphene, DDT, parathion, malathion, endrin, and rotenone have been used for general control. All of these materials are occasionally reported by growers in North Carolina as being ineffective on cabbage loopers. Erratic control also seems to have been the experience of research entomologists. This, plus the fact that most reported research has been done only on the cabbage looper, was a factor in the decision to conduct tests in North Carolina.

Wilcox & Howland (1954) applied dusts to turnips at the rate of 30 pounds per acre. Holes in the leaves caused by cabbage loopers, were reduced 95% with 10% toxaphene and 63% with 5% DDT. Damage on cauliflower was reduced 86% with 10% toxaphene, 59% with 10% DDT, and 24% with 5% DDT. McEwen & Hervey (1956) in tests on DDT-resistant cabbage loopers found that 2 pounds of actual toxaphene per acre gave less control than 1 pound of DDT. Neither material gave practical control. Dieldrin and parathion gave poor control. Endrin, Isodrin, and Phosdrin gave excellent control. Bibby (1957) obtained outstanding control of cabbage loopers with a malathion-Perthane mixture (1 pound of each per acre). Neither malathion at 2 pounds nor toxaphene at 4 pounds per acre gave good control. Toxaphene-DDT mixtures were more effective than either of these materials alone, at low dosages. Parencia *et al.* (1957) found that dieldrin-DDT mixtures gave poor control of the cabbage looper on cotton. Toxaphene-DDT mixtures or endrin gave satisfactory control in the regular cotton spray schedule by airplanes. Wene (1957) applied dusts on pre-head cabbage at the rate of 20 pounds per acre and obtained excellent control of the cabbage looper with 1.5% endrin, 20% toxaphene, and combinations of DDT with malathion or parathion. Malathion was the least effective. On large loopers 20% toxaphene was about the same as 2% Phosdrin or 7.5% malathion. Rotenone gave little control. Toxaphene was very effective on small loopers during cool weather but less effective on large loopers during warm weather.

In 1955 a test was made at Fletcher, North Carolina on small cabbage heads attacked by the imported cabbageworm. Dusts were applied at the rate of 30 pounds per acre. After 1 week the heads were scored on the basis of feeding injury to new growth. Twenty per cent toxaphene gave excellent control, 4% malathion and 10% DDT gave poor control, and 5% methoxychlor and 5% Perthane gave no measurable control. Dills & Odland (1954) found that chlordane and methoxychlor were significantly inferior to a number of other hydrocarbons tested.

In 1957 two tests were conducted at Fletcher, North Carolina. Insecticide dusts were applied into the tops of the plants with single action hand dusters, at the rate of 30 pounds per acre. Each treatment was replicated four times. Each plot in test 1 consisted of one row 40 feet long. Plots in test 2 consisted of two 20-foot rows. Each scoring was based on a sample of 10 plants per plot. Per cent control was calculated by dividing the number of larvae and pupae in treated plants by the number in untreated plants and subtracting this number from 1.00. Heads were torn apart leaf by leaf. Many larvae had penetrated to the head core. The general infestation was unusually high, ranging up to more than 25 larvae per head (total for all species) in untreated plots. Plants were initially treated with insecticide dusts just before the heads were formed. One week later counts were made of cabbage loopers. When the pre-head plants were treated, cabbage loopers were abundant, but only an insignificant number of young imported cabbage-worms were present and no cross-striped cabbageworms were observed. A second application was made about 3 weeks later when heads were at least 4 inches in diameter. The final scoring was made 1 week later. At this time all three species of cabbageworms were counted. These data are shown in table 1.

RESULTS.—The two tests at Fletcher, North Carolina, showed that the 20% toxaphene was more consistently effective against all three species of cabbage worms than the other insecticides tested, at the concentration levels used. Dust containing 2.5% Guthion gave complete control in one pre-head treatment test against the cabbage looper but gave only poor control when the dusts were applied after the heads had formed. One and one-half per cent Phosdrin and 4% Thiodan gave poor to fair control of the cabbage looper and were somewhat more effective in the pre-head treatment tests than in the head treatment tests. Two per cent Sevin[2] ranged from no control of the cabbage looper, in one head treatment test, to poor control in the other test. No pre-head treatment was made with this insecticide.

Sevin gave fair to good control of the cross-striped and imported cabbage worms. All of the other insecticides gave from very good to complete control of these larvae. Reid & Cuthbert (1949) reported a variation between several of the hydrocarbons and their toxicity to different species of cabbage caterpillars.

When the cabbage heads had formed, the larvae gained some protection from insecticide poisoning by burrowing. As they

[1] Accepted for publication January 16, 1958.

[2] N-methyl-1-naphthyl carbamate.

Table 1.—Control of three species of cabbage caterpillars in cabbage with insecticide dusts. Fletcher, North Carolina 1957.

Insecticide[a] and Per Cent Concentration	Per Cent Control 1 Week After Treatment							
	Cabbage Looper (Pre-Head Cabbage)		Cabbage Looper (Head Cabbage)		Imported Cabbageworm (Head Cabbage)		Cross-Striped Cabbageworm (Head Cabbage)	
	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
Guthion, 2.5	100	78	49	40	96	95	100	100
Phosdrin, 1.5	66	78	66	40	100	100	99	100
Sevin, 2	—	—	40	0	88	68	78	93
Thiodan, 4	89	70	58	70	96	93	100	99
Toxaphene, 20	96	89	74	75	99	98	100	100

[a] Applied at the rate of 30 pounds per acre when the plants were in a pre-head stage on July 4 and again on July 25 when the heads were about 4 inches in diameter.

matured many of them went progressively deeper into the heads. This situation probably accounts for a reduction in effectiveness of control and for much of the difficulty experienced by growers, especially when applications are made too late or are too far apart.

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Reactions of Different Populations of *Panonychus ulmi* (Koch) to Various Phosphorus-Based Acaricides

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Any information or observations pertinent to the serious problem of insect or mite resistance to spray chemicals are of value and with this idea in mind, the following situation is reported:

The fact that different populations of insects and/or mites react differently or in different degrees to the same spray chemical is not unknown. However, the clear cut differentiation found among populations of the European red mite (*Panonychus ulmi* (Koch)) scattered over Northern Ohio is in some aspects unique. In the autumn of 1952, rather high populations of the European red mite were noted in a Red Delicious orchard at Clyde, Ohio. As parathion had been used frequently for a period of several years in this planting, resistance was suspected and was confirmed by experimental work in 1953, 1954, and 1955. These results have been published in detail as Research Circular 37 of the Ohio Agricultural Experiment Station. One of the findings from these tests was the fact that this particular mite population was in no way resistant to the phosphorus-based systemic demeton (Systox). This being the case, demeton was recommended as an acaricide for use against European red mite populations that had developed resistance to parathion. Following the recommendation, demeton was successfully used in the Clyde area in 1955, 1956, and 1957. In fact, the results have been so satisfactory that growers plan to use it again in 1958. In 1956 most Ohio growers who had parathion resistant mites used demeton successfully but two reports were received to the contrary. In these cases, details were lacking regarding the treatments and the author was inclined to blame poor spray practice for the results. However, in the spring fo 1957, an orchardist located near Wooster, Ohio, who was using demeton on a parathion resistant population for the first time, reported no success. This led to an experiment in the orchard in which a number of phosphorus-based materials were used along with a number of other acaricides.

The results are given in table 1. Spray applications were made on July 9 and 17. Replicated single tree plots of the variety Red Delicious were used.

The data presented in table 1 show quite clearly that the two phosphorus-based materials were relatively ineffective in controlling this mite population, whereas Kelthane soon reduced it to futile numbers. All this is very much in contrast to the results secured at Clyde, Ohio and published in Research Circular 37.

Table 1.—Control of a parathion-resistant mite population with related and nonrelated acaricides. Wooster, Ohio, 1957.

MATERIAL AND DOSAGE	MITES PER LEAF AND DATES OF COUNTS					
	Pre-Spray 7/8	7/11	7/17	7/20	8/2	8/12
Demeton EC 21%, 4 ozs.—100 gals.	46.6	7.3	60.4	37.1	27.8	29.2
Demeton EC 21%, 8 ozs.—100 gals.	19.5	2.7	33.7	7.0	14.4	21.6
Trithion F4, 8 ozs.—100 gals.	30.7	10.4	31.1	6.7	3.6	9.1
Kelthane EC 25%, 1 pt.—100 gals.	25.5	.5	.3	T[a]	T	0
Check	32.1	21.4	54.3	63.9	32.2	24.2

[a] =Trace or less than 0.1 mite per leaf.

CONCLUSIONS.—It is obvious that in Northern Ohio there are at least two races of parathion-resistant mites. One population is not resistant when demeton is used as a means of control whereas the other, due to unknown factors, immediately becomes or already is resistant to demeton.

That there are strains or races of *Panonychus ulmi* that are intermediate in resistance between the populations cited is indicated by the experience of growers who were successful in controlling mites for one season with demeton before resistance became apparent.

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Tests with Malathion for Hog Lice Control[1]

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Tests were conducted at the University of Maryland in cooperation with the Department of Animal Husbandry to determine the effectiveness of malathion for control of the hog louse, *Haematopinus suis* (L.) Sixteen pigs, averaging 90 pounds, were selected for the test and were kept in a hog lot separate from the other animals. The data on control presented in this note represent the results of one spray application. Malathion emulsion concentrate was diluted with water and applied with a 3-gallon compressed air sprayer. Three concentration levels were used. Four pigs were sprayed with a 0.5% formulation and four were sprayed with 0.25% and four with 0.1% of the active ingredient of malathion. Approximately 475 ml. of spray material were used on each pig. The remaining four pigs were untreated check animals.

As shown in table 1, malathion at a dosage of 0.5% or 0.25% controlled adult hog lice and protected the pigs from lice for more than 9 days. Later, gross observations of a herd of swine sprayed once with 1% malathion (approximately 1 oz. of 50%

[1] Misc. Publ. No. 309, Contribution No. 2869, of the Maryland Agricultural Experiment Station, Department of Entomology. Accepted for publication December 23, 1957.

Table 1.—Results of malathion treatments for the control of hog lice.

Animal No.	Pre-Treatment Infestation (No. of Adults)[a]	Concentration Used (Per Cent)	Post-Treatment Infestation			
			After 9 Days		After 35 Days	
			Adults	Eggs	Adults	Eggs
B4084	100	0.5	None found	None found	None found	Present
Y7	50	.5	None found	None found	[b]	[b]
4069	50	.5	None found	None found	None found	Present
Y6	100	.5	None found	None found	Present	Present
Y8	100	0.25	None found	None found	Present	Present
Y43	100	.25	None found	None found	Present	Present
4068	50	.25	None found	None found	[b]	[b]
Y45	50	.25	None found	None found	None found	Present
Y1	100	0.1	None found	None found	Present	Present
Y42	50	.1	Present	Present	Present	Present
4046	50	.1	None found	None found	[b]	[b]
Y2	100	.1	Present	Present	Present	Present
B6	20	Check	Present	Present	[b]	[b]
B8	50	Check	Present	Present	[b]	[b]
B7	150	Check	Present	Present	[b]	[b]
4047	150	Check	[b]	[b]	Present	Present

[a] Represents approximate counts. Eggs were present on all animals.
[b] Pig too muddy to make accurate records.

emulsion concentrate per gallon of water) indicated that compete control was obtained for more than 30 days after treatment.

To determine whether malathion was safe to apply on pigs being finished for slaughter, two pigs were selected and sprayed with 1% malathion. Two applications were made using 650 ml. of spray material per pig on the first application and 750 ml. on the second application which was made 1 week after the first. No effort was made to keep the spray from the snout, eyes, etc. Three days later these pigs were slaughtered and samples of fat, lean meat and liver tissue were taken for chemical analysis.

Table 2 gives the analytical results from two chemical laboratories. From these data it was concluded that malathion applied as described is both effective and safe for hog lice control.

Table 2.—Residues in swine sprayed with malathion[a] 1956.

Tissue Analyzed	Malathion Recovered		Remarks
	Treated (P.p.m.)	Checks (P.p.m.)	
Fat[b]	None	0.07	Samples held in freezer until analyzed May 12.
Lean meat[b]	None	.05	
Liver[b]	0.04	.08	
Fat[c]	None	See remarks	0.9 p.p.m. Malathion added to check samples of fat. Recoveries averaged 93.5%
Lean meat[c]	None		
Liver[c]	None		

[a] 1% emulsion concentrate spray applied; 650 ml. per animal on April 21; 750 ml. per animal on April 28. Samples taken May 1.
[b] Analyzed by F. P. Veitch, Dept. of Chemistry, University of Maryland.
[c] Analyzed by R. H. Carter, Pesticide Chemicals Research Section, U. S. Dept. of Agriculture.

The Residual Toxicity of Insecticides to *Scolytus multistriatus*[1]

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The smaller European elm bark beetle, *Scolytus multistriatus* (Marsh.), is one of the principal vectors of *Ceratocystis ulmi* (Buism.) Moreau, the causal fungus of Dutch elm disease. Infection from beetle feeding is reduced both by protection of healthy trees with DDT sprays and by destruction of infected trees to prevent beetle attack or emergence. The healthy elms are most susceptible to infection by vector feeding from the end of the dormant season until mid-July (Parker *et al.* 1941). Sprays are applied just before bud break and must have residual toxicity for at least this period. In addition to DDT, Matthysse *et al.* (1954) have shown that dieldrin is effective as a residual insecticide against the native elm bark beetle, *Hylurgopinus rufipes* (Eichh.).

This paper reports on the residual behavior of four insecticides compared with the standard, DDT.

MATERIALS AND METHODS.—Emulsions of DDT, dieldrin, heptachlor, lindane and malathion were applied to American elms before leaf bud break and the residues weathered under field conditions. Four trees, 10 to 12 feet high were sprayed to the point of run-off with each insecticide concentration. The sprays were applied with a 3-gallon Hudson sprayer fitted with an extension that would reach the tops of the trees.

The residues were bioassayed at 4, 8 and 13 weeks after treatment using *Scolytus multistriatus* as the test insect. On the day of each assay twigs were clipped from the trees and brought into the laboratory in paper bags. Most of the leaves were removed and the twigs were cut to fit into wide-mouth gallon jars. The beetles show a marked preference for twig crotch feeding so approximately equal numbers of twig crotches were used in each jar. Beetles reared in the insectary from bark collected in the field were placed in each jar and mortality counts were recorded at 24 and 48 hours. Counts of the amount of feeding or "scores" were made at 48 hours. Any feeding mark that penetrated to the xylem surface was termed a score. Since the fungus must reach the xylem surface to infect the tree the efficiency of the insecticides in preventing scores was taken as indirect evidence that the transmission of the disease would be prevented.

The bioassay 4 weeks after treatment was conducted at 72° F. using 25 beetles and 35 twig crotches per jar while the 8- and 13-week tests were run at 78° F. and utilized 10 beetles and 23 twig crotches per jar.

[1] Accepted for publication January 2, 1958.

RESULTS AND DISCUSSION.—Table 1 presents bark beetle mortality after 24 hours exposure to treated twigs and the amount of scoring during 48 hours expressed as per cent of the number of twig crotches or feeding sites available per jar. None of the insecticides tested was more efficient than DDT in preventing feeding. The 1% and 2% DDT emulsions both gave about equal protection. The per cent kill of beetles exposed to twigs treated with 0.5% and 1% dieldrin sprays was equal to or higher than the kill with DDT. However, the ability of dieldrin to produce a high mortality at 24 hours was not reflected in a lower incidence of feeding scars. Both the 0.5% and 1% levels of dieldrin allowed a disproportionate amount of scoring. The 0.25% dieldrin treatment was ineffective.

Table 1.—Comparative efficiency of various insecticides expressed as per cent mortality of *Scolytus multistriatus* adults at 24 hours exposure to treated twigs and the per cent of twig crotches with feeding scores that reached the xylem area at 48 hours.

INSECTICIDE	BEETLE MORTALITY			SCORES		
	4 Wks.[a]	8 Wks. Assay	13 Wks.	4 Wks.	8 Wks. Assay	13 Wks.
DDT 1%	72	92	76	0	15	4
DDT 2%	81	100	77	5	12	4
Dieldrin 0.25%	50	59	64	73	49	36
Dieldrin 0.5%	82	89	89	22	29	23
Dieldrin 1%	96	94	90	11	21	31
Heptachlor 0.5%	73	20	35	27	67	46
Lindane 0.5%	54	33	43	56	60	22
Lindane 1%	77	74	60	3	15	10
Malathion 0.5%	41	11	31	50	56	38
Malathion 1%	64	18	29	20	47	33
No treatment	16	32	31	69	65	62

[a] Twigs for bioassay were collected from treated trees at 4, 8, and 13 weeks after treatment.

Residues of 1% lindane spray persisted very well and compared favorably with DDT in prevention of scoring. The tenacity of lindane residues was remarkable in view of the volatile nature of this insecticide and may possibly be explained by penetration into the bark. Matthysse *et al.* (1954) commented on this long residual life where lindane was used in twig dipping experiments.

Residues of 0.5% heptachlor, 0.5 and 1% malathion and 0.5% lindane sprays were largely ineffective in preventing the beetles from feeding.

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Malathion and Parathion with Oil for Control of California Red Scale[1]

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Combinations of parathion and oil have had extensive use for control of the California red scale, *Aonidiella aurantii* (Mask.), on citrus in California. One gallon of oil plus 1 pound of 25% parathion and 1⅜ to 1¾ gallons of oil plus ½ pound of 25% parathion per 100 gallons of spray are the two mixtures that are recommended.[3] The first is more effective against the red scale, but the second is better for use against the citrus red mite, *Metatetranychus citri* (McG.), and since it is also a good scale treatment (Cressman *et al.* 1954) it is the more commonly used.

An important objection to the oil-parathion sprays is their phytotoxicity. Oranges are more susceptible than lemons, and after some injury to oranges in the early work the principal use of these sprays has been on lemons. Malathion is less toxic to both mammals and plants when applied with oil. Carman (1956) found that the addition of malathion consistently increased the effectiveness of oil sprays. The mixture of 1⅜ to 1¾ gallons of oil plus 1 pound of 25% malathion is a recommended treatment for Valencia oranges and lemons and has had considerable commercial use. This report gives the results of two field experiments started in 1952 and 1954 near Whittier, Calif., in which selected concentrations of oil plus parathion and oil plus malathion were compared.

MATERIALS.—A malathion wettable powder was used in both experiments, and parathion wettable powder in the first and emulsifiable concentrate in the second. The special light oil had the following specifications: Viscosity (100° F.) 51 seconds Saybolt, unsulfonatable residue 91%, distillation (636° F.) 82%. The light medium oil conformed to the specifications of the California Department of Agriculture (Cox 1938).

FIRST EXPERIMENT.—On April 29, 1952, heavily infested lemon trees near Brea were manually sprayed with 1 gallon of special light oil plus ¾ pound of 25% parathion or 1½ pounds of 25% malathion per 100 gallons. Three eight-tree plots were sprayed with each combination. Two months later mortality counts were made of 300 adult female scales on the branches on six trees per plot. The average kill from the oil-parathion spray was 96.3% and from the oil-malathion spray 88.8%. The difference was significant.

In September all plots were treated with parathion and ovex. No counts on infestation were made then, but inspection showed more scale buildup in the malathion plots.

Citrus red mites on the foliage were estimated by the procedure described by Henderson & McBurnie (1943). The numbers of mites per 12-leaf sample on three dates were as follows:

	Oil Plus Malathion	*Oil Plus Parathion*
July 9	1.7	0.9
July 28	8.7	10.0
August 20	27.2	28.5

The period of control was rather short, and there was no difference between the two treatments. There were no adverse tree reactions.

SECOND EXPERIMENT.—In 1954 another experiment was begun, with 1.8 gallons of light medium oil plus ½ pound of 25% parathion or 1 pound of 25% malathion per 100 gallons. Sprays were applied to lemons at Corona with an oscillating-boom unit with a high-speed centrifugal pump. Three plots five or six rows wide and 22 trees long were treated with oil plus parathion and two with oil plus malathion. Sprays were applied on October 6, 1954, and November 11, 1955. Red scale and mite counts were made on 10 of the inside trees in each plot. Effectiveness against scales was measured by estimating the infestation before treatment and again at intervals of approximately a year. Methods of making the scale counts were similar to those described by Cressman & Broadbent (1944). The number of adult female scales on 40 fruits of each tree was recorded.

[1] Accepted for publication January 23, 1958.

[2] B. M. Broadbent (retired October 31, 1955) and Francis Munger assisted in these experiments.

[3] 1957 Spray Program for California Citrus Fruits, University of California Citrus Experiment Station and Agricultural Extension Service.

The numbers of scales per fruit were as follows:

	Oil Plus Malathion	*Oil Plus Parathion*
August 30, 1954	1.9	1.5
August 20, 1955	2.5	1.2
September 21, 1956	6.8	1.1

The difference between materials was significant. The pretreatment infestation in the oil-malathion plots was slightly higher than in the oil-parathion, but the ratios of the final infestations to the pretreatment populations for the two combinations were 3.7 and 0.7. The infestation in the oil-malathion plots increased each year while that in the oil-parathion plots declined slightly. The infestation in the oil-malathion plots showed a marked increase in the second year but not in the oil-parathion plots. This may have been due to the longer interval between the 1955 and 1956 counts and weather very favorable for red scale in the late summer and fall of 1955.

A rather heavy infestation of citrus red mites developed in the spring of 1955. Leaf samples taken on June 13 showed an average of seven mites per leaf, with no difference between treatments. No mite infestation developed in the year after the 1955 applications. The increase in amount and weight of the oil was accompanied by better mite control than was obtained in the first experiment.

Leaf drop was never more than the moderate one often following the use of oil alone, and there was no difference between treatments.

DISCUSSION.—There was no evidence of phytotoxicity in these experiments, but addition of parathion to oil has sometimes increased leaf and fruit drop in other experiments and in commercial applications. Malathion has not had this effect. On heavy red scale infestations oil plus malathion was not quite equal to oil plus parathion at the dosages used, but the advantages of malathion in respect to both mammalian and plant toxicity are such that it would probably be the preferred material for use with oil where combined control of California red scale and citrus red mite is the objective. However, in lemon groves with a severe scale infestation or a history of difficulty in control, the oil-parathion sprays should be considered.

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The Effectiveness of Several Insecticides for Control of Insects on Snap Beans[1]

L. P. DITMAN and ROBERT C. WILEY[2]

During the 1957 season 9 insecticides were tested in the field against insect pests of snap beans. Among the insecticides used were malathion and Dilan, which have been recommended for control of bean insects in Maryland for several years; two systemic chemicals, demeton and Thimet, and several promising newer products.

PROCEDURE.—All insecticides were applied in emulsion form in water at the rate of 25 gallons per acre with a "Slosser" type boom. All treatments were replicated four times on four-row plots in a randomized block arrangement. Crop yields, extent of pod injuries, Mexican bean beetle (*Epilachna varivestis* Muls.) injury to plants, and estimates of potato leafhopper (*Empoasca fabae* (Harr.)) populations were recorded, usually from the two middle rows of each plot. The Top Crop variety of snap beans was used in these tests, and was planted July 2. The non-systemic insecticides were applied on July 24, August 1, August 7, and August 15. One application of the systemic insecticide Thimet was applied to the foliage July 10 in a water emulsion at the rate of 9 ounces of the active ingredient per acre. On July 24 one application of demeton was made in the same manner but at the rate of 12 ounces of the active ingredient per acre. Beans were harvested on August 20, 27, and September 12. Samples of beans from each plot from the first and second harvests were canned for taste evaluation. Adult leafhopper population counts of each plot were made by the sweep method on August 2 and nymphal leafhopper counts (average number per 25 leaves from each plot) were made on September 4. Estimates of Mexican bean beetle damage, by ratings based on percentage of plants attacked by adult and larval stages, were made on September 4. Plot damage was rated on a scale of 0 to 3 as follows: 0, indicating no injury; 1, less than 25% of plants injured; 2, from 25% to 75% of plants injured; and 3, from 75% to 100% of plants injured. Small feeding areas were attributed to adult beetles and large areas of damage were attributed to larval feeding. At this time the immature stages were present and were used to confirm larval feeding areas.

RESULTS AND DISCUSSION.—The nature of treatments, crop yields, and data pertaining to insect control are given in table 1.

At the dosages applied all insecticides used except demeton and Thimet gave considerable control of the Mexican bean beetle on the basis of plant injury. Parathion, Diazinon, Sevin,[3] Trithion, and Thiodan gave results comparable to malathion and Dilan. There appeared to be a greater difference in the performance of the different insecticides against the potato leafhopper than against the Mexican bean beetle. At the time adult populations were estimated there was a heavy migration of adults into the field so that there were insects present on all plots, resulting in a partial masking of the effectiveness of treatments. It is believed that the presence of nymphs indicates the ability of the species to survive on treated plants and is a more accurate index of the effectiveness of the treatment. Only on Dilan-treated plots were no immature leafhoppers found, but a high degree of effectiveness is indicated also for Trithion, Thiodan, and Sevin. Differences between percentages of scarred pods and pods injured by corn earworm (*Heliothis zea* (Boddie)) and other boring larvae for the various treatments are not significant. The earworm population was too low to evaluate and there was too much variation in percentage of scars within treatments. Because of unevenness in germination of seed resulting from variation in soil moisture following planting, yield differences between treatments are not significant (F-value, 0.91; required @ 5% 2.16). Differences between blocks, incidentally, were highly significant (F-value, 7.36; required at 1%, 4.51) and indicate the great lack of uniformity between blocks. There was no significant difference in the flavors of canned beans from the different treatments (F-value, 0.6; required at 5%, 1.8) though beans of the first harvest were better flavored than beans from the second harvest.

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[2] L. P. Ditman, Research Professor, Dept. of Entomology; R. E. Wiley, Assistant Professor, Dept. of Horticulture, University of Maryland Agricultural Experiment Station, College Park.

[3] N-methyl-1-naphthyl carbamate, also called CC7744.

Table 1.—Results of insecticides applied to snap beans in 1957 for control of potato leafhopper and Mexican bean beetle.

Insecticide and Dosage per Acre[a]	Average Yield per Plot (Lb.)	Per Cent of Pods		Potato Leafhopper		Rating of Mexican Bean Beetle Injury, September 4		Flavor Rating[b]	
		Scarred	Injured by Earworm	Adults per Sweep Aug. 2	Nymphs per 25 Leaves Sept. 4	By Adults	By Larvae	Aug. 20	Aug. 27
Parathion, 1 pt.	47.8	1.5	0	2.5	1.7	1.0	1.0	4.06	3.84
Malathion, 1 qt.	39.2	4.0	0.5	1.5	1.5	1.0	1.0	3.91	3.94
Trimet, 1.12 pts.	46.6	6.0	2.2	6.3	3.2	2.5	2.3	4.06	3.91
Diazinon, 1 qt.	42.8	2.0	.5	2.1	1.0	1.0	1.0	4.03	3.72
Demeton, 1.5 qts.	47.8	3.7	.2	5.5	1.5	2.0	2.2	4.22	3.63
Untreated	39.0	4.7	.7	6.5	5.5	2.2	2.5	4.16	3.94
Sevin, 1 gal.	36.1	4.7	.0	1.2	0.2	0.7	0.7	3.97	3.75
Trithion, 1 pt.	49.6	2.0	.2	1.7	0.5	1.0	0.7	4.16	3.94
Thiodan, 1 qt.	47.3	3.7	.0	1.7	0.2	1.0	1.2	4.22	3.50
Dilan, 1 qt.	44.7	2.7	.2	2.0	0.0	1.0	1.0	4.13	3.72

[a] Pounds of actual toxicant in each gallon of the concentrates used: parathion, 2; malathion, 4; Thimet, 4; Diazinon, 2; demeton, 2; Sevin, 1; Trithion, 4; Thiodan, 2; and Dilan, 2.

[b] Flavor ratings: 1—off-flavor, not acceptable; 2—slightly off-flavor; 3—below standard, no detectable off-flavor; 4—equal to standard in flavor; 5—better than standard in flavor.

Field Tests of Materials for Control of the Cotton Leafworm[1]

PERRY L. ADKISSON,[2] *Southeast Missouri Research Center, Sikeston*

The cotton leafworm, *Alabama argillacea* (Hbn.), has often been a serious pest in southeast Missouri (Enns 1951). Outbreaks in the fall are frequently widespread but sporadic and often catch many farmers and insecticide dealers by surprise. The problem is not the failure of recommended control measures but rather of having an inadequate supply of insecticides available for immediate use late in the season. Serious losses can occur if there are sudden outbreaks involving large acreages and if the stocks of standard insecticides in the immediate area have been nearly depleted. For this reason, the toxicity of various new insecticides to the cotton leafworm should be known in order that every effective insecticide can be used in these emergencies.

PROCEDURE.—Tests were conducted on unreplicated plots 16 rows wide and 300 feet long. Leafworm counts were made by taking 10 sweeps with an insect net at each of three random locations within each plot. The net used was a standard insect sweeping net 12 inches in diameter. The first worm count was made just prior to application of insecticides. The second count was made 48 hours after application.

The insecticides were applied as sprays prepared from emulsifiable concentrates. Applications were made by a high clearance sprayer using one nozzle per row. The sprayer was calibrated to deliver 2 gallons of spray per acre. Insecticides tested and dosage in terms of pounds of active ingredient per acre were: Guthion, 0.25; toxaphene, 2.0; endrin, 0.20; Sevin, 0.50 and 1.0; Monsanto CP7769,[3] 0.25; malathion, 0.50 and 1.0; Hercules AC-528, 0.25 and 0.50; and Phosdrin, 0.25.

RESULTS.—Results are presented in table 1. All materials tested gave satisfactory control within the 48-hour test period, although some appeared to give better control than others. Hercules AC-528 gave excellent control but had a slower killing action than any of the other materials.

Table 1.—Effectiveness of several materials tested for cotton leafworm control in Missouri, September, 1957.

Treatments	Pounds/ Acre	No. Worms per 30 Sweeps		
		Before Spray	48 Hours after Spray	Per Cent Mortality
Check	—	121	155	—
Guthion	0.25	121	6	95.0
Toxaphene	2.00	125	9	92.8
Endrin	0.20	153	0	100.0
Sevin[a]	0.50	185	6	96.8
Sevin	1.00	138	2	98.6
CP7769	0.25	148	18	87.9
Malathion	0.50	163	0	100.0
Malathion	1.00	172	0	100.0
Hercules AC-528	0.25	149	1	99.4
Hercules AC-528	0.50	198	0	100.0
Phosdrin	0.25	176	0	100.0

[a] N-methyl-1-naphthyl carbamate.

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[1] Contribution from Missouri Agricultural Experiment Station, Journal Series No. 1832. Approved by Director. Accepted for publication January 13, 1958.

[2] Assistant Professor, Department of Entomology.

[3] Ethylthyiomethlidynetri(phosphonic acid diethyl ester).

Parasitic Mites (Acarina: Mesostigmata) from Alabama Mammals[1]

KIRBY L. HAYS and F. E. GUYTON, *Agricultural Experiment Station, Alabama Polytechnic Institute, Auburn*

Mesostigmatic mites have been implicated as vectors of virus and rickettsial disease agents, as well as intermediate hosts of protozoa and nematode parasistes of birds, mammals, and reptiles. For this reason, it is desirable to know the species of mites parasitizing animals in certain localities. Therefore, during 1953 and 1954 small mammals and their ectoparasites were collected in Lee County, Alabama. Lee County is located in an ecologically varied area in the east-central part of the state. The soils of the northern part of the county are typical red clays of the Piedmont Plateau, while those of the southern part of the county are deep sands of the Lower Coastal Plain. Parts of the

[1] Accepted for publication January 9, 1958.

county are cultivated; however, much of the area is in second growth pine or hardwood forests, and large areas of waste land are in evidence. Several large swamps and streams that cross the county change and influence the local ecology. Therefore, a variety of habitats is available for mammalian use.

Two hundred and ninety-seven small mammals of 30 species were collected in this area. Two hundred and fifty-nine of these animals were examined for acarian parasites. One hundred and fifty-seven were found to be parasitized by mites. A total of 1949 mites of 13 species were collected, mounted on slides, and identified. Specimens of each species are in the collections of the senior author and the Alabama Polytechnic Institute.

The authors wish to thank Drs. Harry D. Pratt and John Lane, Communicable Disease Center, Atlanta, Georgia, for identifying species about which the authors were in doubt. The list of mammalian hosts and their ectoparasitic mites follows:

Species of Mammal	No. Collected	Ectoparasite	Average No. Per Host
	DEDELPHIIDAE		
Didelphis virginiana Kerr	2	*Ornithonyssus bacoti* (Hirst)	1.5
		Ichoronyssus wernecki (Fonseca)	6
	TALPIDAE		
Scalopus aquaticus howelli Jackson	8	*Haemogamassus harperi* Keegan	1.5
		Haemolaelaps glasgowi (Ewing)	62
	SORICIDAE		
Blarina brevicauda carolinaensis (Bachman)	1	*Eulaelaps stabularis* (Koch)	1
		Haemogamassus harperi Keegan	4
		Haemolaelaps glasgowi (Ewing)	2
Cryptotis parva (Say)	2	None	
	VERSPERTILIONIDAE		
Lasionycteris noctivagans (LeConte)	2	None	
Myotis lucifugus lucifugus (LeConte)	6	*Steatonyssus certatognathus* (Ewing)	3
Nycteris borealis borealis (Muller)	1	*Steatonyssus ceratognathus* (Ewing)	2
Nycticeius humeralis	1	*Steatonyssus ceratognathus* (Ewing)	1
	MOLOSSIDAE		
Tadarida cynocephala (LeConte)	4	*Steatonyssus ceratognathus* (Ewing)	4
	PROCYONIDAE		
Procyon lotor lotor (L.)	2	None	
	MUSTELIDAE		
Mephitis elongata (Bangs)	1	None	
Spilogale putorius (L.)	2	None	
	CRICETIDAE		
Ondatra zibethica zibethica (L.)	1	*Laelaps multispinosus* Banks	7
Oryzomys palustris palustris (Harlan)	1	None	
Peromyscus gossypinus (LeConte)	8	*Haemolaelaps glasgowi* (Ewing)	1.5
		Eulaelaps stabularis (Koch)	.12
		Haemogamassus liponyssoides (Ewing)	.12
Peromyscus leucopus leucopus (Refinesque)	10	*Haemolaelaps glasgowi* (Ewing)	1.9
Peromyscus nuttalli aureolus (Audubon and Bachman)	7	*Haemolaelaps glasgowi* (Ewing)	4
		Eulaelaps stabularis (Koch)	3
		Ornithonyssus bacoti (Hirst)	1.3
Peromyscus polinotus (Subspecies?)	5	*Haemolaelaps glasgowi* (Ewing)	.4
Pitymys pinetorum pinetorum (LeConte)	5	*Haemolaelaps glasgowi* (Ewing)	2
		Laelaps kochi Oudemans	2
Reithrodontomys humilis humilis (Bachman)	5	*Haemolaelaps glasgowi* (Ewing)	1.2
		Eulaelaps stabularis (Koch)	.2
Sigmodon hispidus hispidus Say and Ord	132	*Haemolaelaps glasgowi* (Ewing)	30
		Eulaelaps stabularis (Koch)	.06
		Haemogamassus liponyssoides (Ewing)	.06
		Ornithonyssus bacoti (Hirst)	1.9
	MURIDAE		
Mus musculus musculus (L.)	15	*Haemolaelaps glasgowi* (Ewing)	1.4
Rattus norvegicus (Erxleben)	8	*Laelaps nuttalli* Hirst	13
		Laelaps echidninus (Berlese)	.9
		Ornithonyssus bacoti (Hirst)	.5
		Haemolaelaps glasgowi (Ewing)	2
		Ichoronyssus wernecki (Fonseca)	.4
Rattus rattus rattus (L.)	3	*Laelaps nuttalli* Hirst	1.4
		Laelaps echidninus Berlese	.67
		Haemolaelaps glasgowi (Ewing)	3
	GEOMYIDAE		
Geomys tuza mobilensis (Merriam)	1	*Haemolaelaps geomys* Strandtmann	23
	SCIURIDAE		
Sciurus carolinensis carolinensis Gmelin	8	None	
Tamias striatus venustus Bangs	5	*Haemolaelaps glasgowi* (Ewing)	1.4
	PETAURISTIDAE		
Glaucomys volans saturatus Howell	5	*Cosmolaelaps gurabensis* Fox	1.8
	LEPORIDAE		
Sylvilagus aquaticus aquaticus (Bachman)	3	None	
Sylvilagus floridanus floridanus (Thomas)	5	None	

A Device for Rapid Application of Measured Volumes of Toxicants to Individual Insects[1]

B. L. OWEN[2] and L. J. HAYNES,[3,4] *Alabama Polytechnic Institute, Auburn*

Trevan (1922) described a micrometer-controlled syringe for measuring small quantities of liquids. Although somewhat modified by various workers, this instrument remained basically the same for a number of years and was standard in entomology laboratories for application of toxicants to individual insects. In recent years, devices for more rapid application of known amounts of toxicants to individual insects have been developed, such as those described by Dutky & Fest (1942), Bruce (1949), Buck (1949), Ludvik (1953), Roan & Maeda (1953), and Osmun (1957).

Toxicological studies at this laboratory resulted in the need for an instrument for application of toxicants to individual insects that not only was rapid, but also was of rugged construction with a minimum of moving parts. The machine described here was developed to fill these needs.

The device (fig. 1) utilizes a ratchet assembly similar in principle to some of the instruments mentioned above. The body is constructed of $\frac{3}{4}$-inch aluminum alloy, the base of $\frac{1}{4}$-inch aluminum alloy. The micrometer spindle, ratchet, and set pins are of steel fabrication. Construction details are shown in figure 2.

Toxicants are applied topically with a 26-gauge hypodermic needle bent downward at 90° angle from the horizontal, ground flat at the tip and attached to a 0.25-cc. tuberculin syringe. An unmodified needle is used for injection. The movement of the syringe plunger is controlled by a micrometer spindle. This spindle is propelled by a nut that in turn is activated by a ratchet lever that projects above the nut. Accurate control of the lever movement is accomplished by use of two adjustable stop pins attached to the body of the device. The nut has ratchet teeth on one edge of its surface and a scale calibrated in thousandths of an inch on the other. This scale enables the operator to reset and check the metering accurately at any time. The spindle may be returned to the starting position by rotating its knurled end after depressing the spring-loaded key. This key fits into the keyway of the spindle to prevent its rotation.

In most studies at this laboratory, about 1 microliter is the desired volume for application to insects. Advancing the micrometer spindle 0.005 inch delivers approximately this volume when a 0.25-cc. glass syringe is used. Because of slight variation in syringes, each was calibrated with the instrument before using it.

[1] Accepted for publication January 31, 1958.
[2] Department of Zoology-Entomology, Agricultural Experiment Station.
[3] Department of Engineering Shops.
[4] Substantial contributions to the development of the device were made by R. A. Patterson, Department of Engineering Shops.

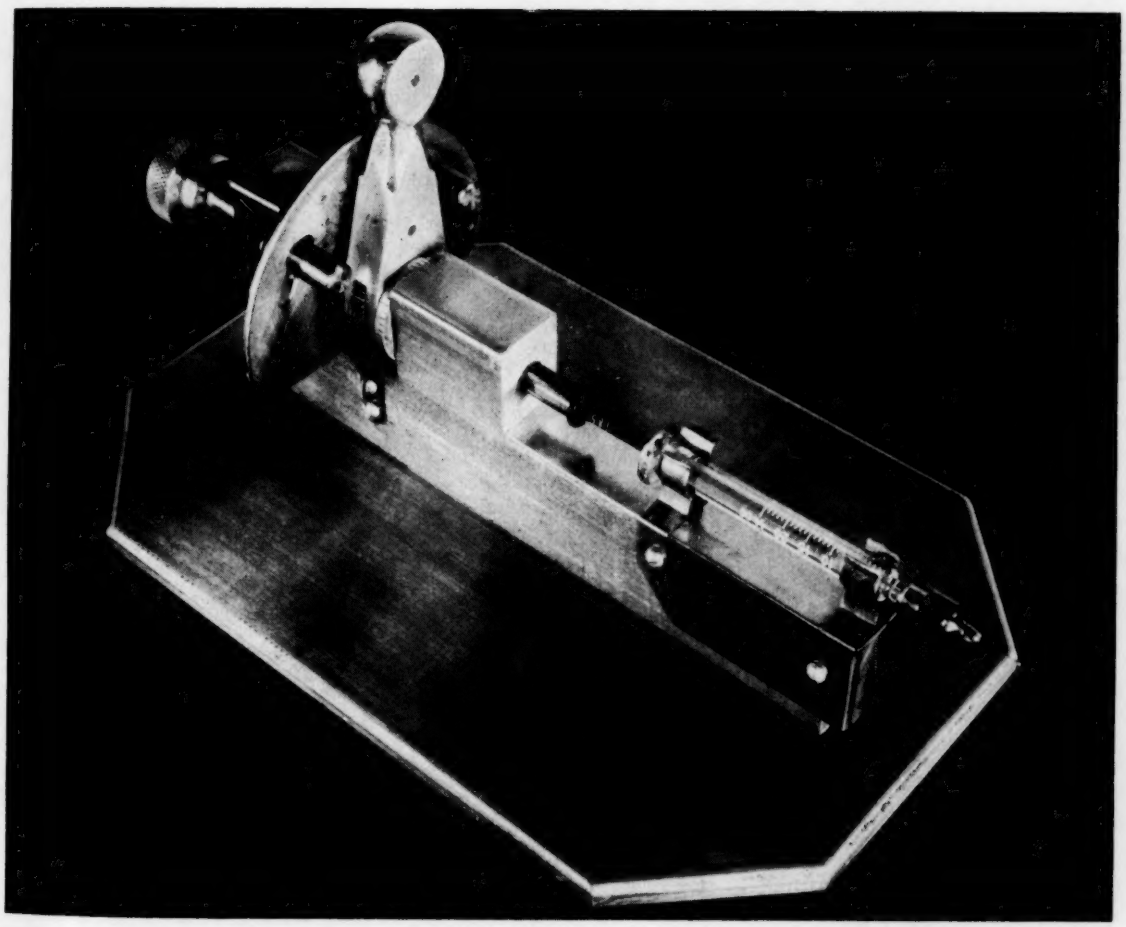

FIG. 1.—Micrometer-syringe device for rapid application of toxicants to individual insects.

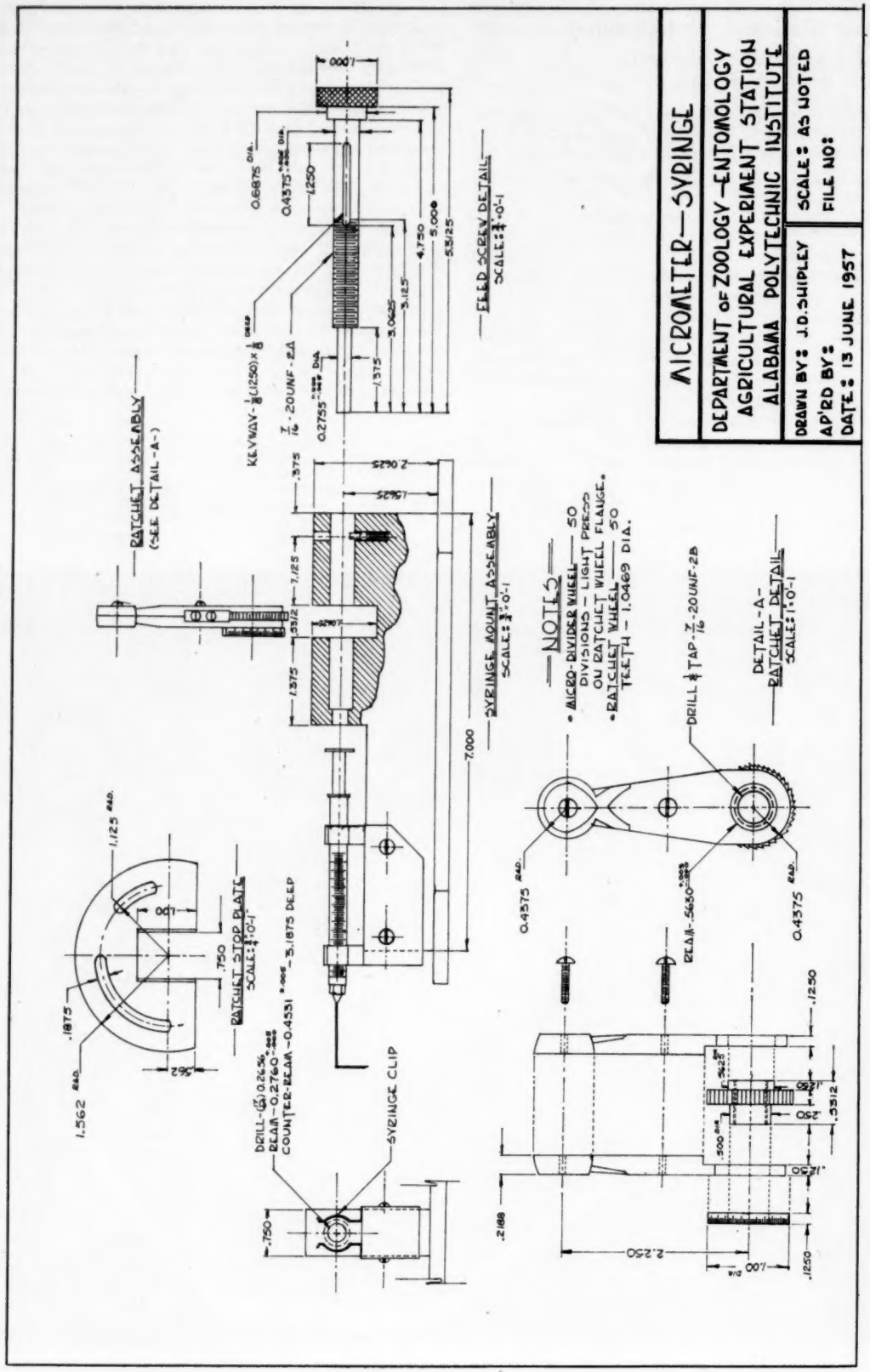

FIG. 2.—Construction details of micrometer-syringe device.

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Effect of the Winter Temperatures of 1956-57 on the Survival Rate of European Red Mite Eggs[1]

S. E. LIENK and P. J. CHAPMAN, *New York State Agriculture Experiment Station, Geneva*

Apple wood heavily infested with winter eggs of the European red mite, *Panonychus ulmi* (Koch), was collected in early March of 1957 for some toxicological studies. A preliminary check showed an abnormally low rate of egg hatching in this material. The unusually cold weather which prevailed during the preceding January appeared to be the most likely cause of this condition. To determine whether or not this was the case, a small-scale survey was initiated. Samples of infested wood were collected from localities representing a considerable range in minimum winter temperatures and the egg hatch was determined on each lot under laboratory conditions.

Wood samples were collected over the period April 9–19. Test material was held under outdoor temperatures for a day or two until egg counts could be made. Counted twig sections were then impaled on thin nails which had been driven through 3×4 inch "squares" of plywood, or the technique described by Chapman & Pearce (1949). Following counting, the eggs were placed in a rearing room where the temperature was maintained at 70° to 80° F. and a relative humidity of about 30%. The data obtained are shown in table 1. Egg hatch in the field started at Geneva on April 30.

The hatching rate in all samples was somewhat sub-normal. This fact was verified in sub-samples of the same lots which had been held under outdoor conditions until a few days before the start of hatching in the field. Both the dry air of the rearing room and the suddenness with which the eggs were exposed to high temperatures are believed to have contributed to the reduced hatching rate. However, since all material was held under the same conditions, the differences noted are relative and probably the essential facts are not obscured.

Collections of wood samples, for the most part, were made on farms near official weather stations. Attention is called to the two records for the North Rose area. The Rhode Island Greening planting was located on a slope rising above Sodus Bay; the Northern Spy block was some 3 miles distant and situated in an air pocket. It is generally appreciated that temperatures tend to reach lower levels and to hold for longer periods in low-lying areas than where air drainage is good as in the Sodus Bay tract.

Table 1.—Apparent influence of the winter temperatures of 1956–57 on the mortality of winter eggs of the European red mite.

New York Locality	Fruit and Variety	Minimum F. Temperature Jan. 15, 1957	Number of Eggs Observed	Per Cent Egg Hatch
Lewiston	Prune, Fellenberg	− 9°	3538	42.2
Barker	Apple, McIntosh	−15°	3171	41.6
Gasport	Apple, King	−18°	3536	41.6
North Rose	Apple, R. I. Greening	−22°[a]	8660	21.04
North Rose	Apple, Northern Spy	−26°	5185	4.56
Geneva	Apple, Baldwin	−25°	5563	4.86
Sodus	Prune, Stanley	−28°	7518	0.4
Peru	Apple, Red Delicious	−34°	1412	2.7
Peru	Apple, Red Delicious	−34°	7570	0.18

[a] Estimated.

It should also be noted that the cold weather which prevailed in New York in midJanuary 1957 held over a 3- to 5-day period. In Geneva, for example, the minimum readings were −5°, −25°, −23°, 0° F. for January 14 to 17 inclusive. Observations made in areas in western New York where the temperatures dropped to −25° to −28° F. showed very little early European red mite activity. By late July or early August, however, sizeable populations developed. In the case of the Rhode Island Greening orchard situated above Sodus Bay, the rate of hatch was fair at the highest elevation gradually diminishing down the slope. This population differential gradually disappeared as the season progressed.

The observation made in 1957 suggests that winter eggs of the European red mite are not adjusted locally to survive the abnormally low temperatures that prevailed during the winter of 1956–57. Considering Geneva as typical of the western New York fruit district, it appears that eggs in this area can withstand the usual low winter temperatures of −5° to −10° F. (the minimum winter temperature for Geneva over the past 35 years, based on local U. S. Weather Bureau records, has ranged from −1° to −31° F. with an average of −6° F.). Any considerable departure from this minimum can apparently be expected to kill many eggs with a temperature of about −22° F. being pivotal. Thus, for districts having climates similar to Geneva one may expect an appreciable killing of European red mite eggs when the winter temperature falls to about −22° F., with the mortality increasing rapidly as the temperature falls below this point.

Although the data presented herein are too limited to draw definite conclusions, the higher than expected survival rate in one of the two Peru, New York samples suggests that eggs in this district may have been conditioned to withstand lower winter temperatures than in warmer portions of the state. The 19-year average winter minimum temperature for Peru, which lies in the northernmost portion of the state, is −21.9° F.

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[1] Journal Paper No. 1103 New York State Agricultural Experiment Station, Geneva. Accepted for publication January 30, 1958.

EDITORIAL

The Forest Entomologist—Forester or Entomologist?[1]

The practice of economic entomology—the control of insect pests of food and fiber crops, livestock, and of the human environment—has increased many fold as the use of agricultural lands and the protection of natural vegetative resources in recent years has become increasingly intensified. One of its important branches is forest entomology, which began to develop in the United States about the end of the 19th century when the need for protecting the country's forests and the increasing volume of wood products from insect damage was recognized.

As in other branches of economic entomology, the formative years of forest entomology were devoted to investigations of the classification and biology of the more obvious insect pests. Gradually, emphasis shifted to the development of applied control methods. Along with this shifting, the investigations tended to be directed more specifically toward the problems of forestry than to those traditionally associated with the science of entomology. As their work veered away from the strictly zoological problems, a large number of practicing American forest entomologists lost some of the working relationships with their fellow economic entomologists. It is the purpose here to trace briefly the more manifest projection of forest entomologists into the field of forestry and to point out some roles that will continue to identify them as zoological scientists.

From the beginning, forest entomologists have followed three major lines of endeavor: (1) entomological research involving the describing of new insect species, the study of their life histories and habits, and the developing and testing of applied control and prevention of insect outbreaks; (2) surveys to detect and appraise the seriousness of outbreaks; and (3) advice and assistance to federal, state, and private timberland owners or managing agencies on the proper use of control techniques.

Much of this work was typically entomological in nature, but much of it also required basic understanding of certain aspects of forestry. Applied control measures for some forest insects such as those for the spruce budworm (*Choristoneura fumiferana* (Clem.)) in the balsam-spruce forests of the New England States and for the western pine beetle (*Dendroctonus brevicomis* Lec.) in the ponderosa pine forests of the West were built by forest entomologists upon the recognized silvicultural and forest management techniques of the forestry profession. Furthermore, most forest insect surveys in the United States during past years were not insect surveys *per se*, but surveys of insect damage that involved the recognition and measurement of infested or insect-killed trees or forest acreages. Such surveys required a working knowledge of forest mensuration and of forest surveying and mapping. Until recently, forest entomologists frequently used survey results to interpret insect control needs in terms of the economic damage or threat of damage to the forest resource. This interpretation could only be made with some of the forester's knowledge of the economic components used in appraising timber stumpage value. These tasks were efficiently undertaken because many forest entomologists until the present time have held degrees in forestry as well as in entomology.

With these responsibilities, the forest entomologist became part botanical scientist and part zoological scientist—part forester, part entomologist. The situation compounded the problems of education, of in-service training, and of the maintenance of contacts with professional societies representing both sciences. Employing agencies often found it difficult in past years to find recruits adequately trained in forestry as well as in entomology primarily because colleges and universities found certain hindrances in integrating the two curriculums.

Many of the scientific papers by forest entomologists have appeared in either forestry or entomological journals depending upon subject emphasis. These writers have belonged to either forestry or entomological societies or to both where, all too often, they have experienced difficulty in maintaining professional communication with their fellow members. The environment and insect problems of the forest, for instance, have often been little understood by economic entomologists engaged mostly in agricultural crop, livestock, or public health entomology. Likewise, foresters often have failed to recognize biological factors inextricably associated with the epidemiology of forest insect infestations, sometimes even to unwittingly fostering insect epidemics in the very resource they professed to protect.

Like most other new professions, forest entomology advanced by a process of constant adjustment to enable it to find its rightful place in the family of sciences. With each passing year it became more definitive and its functions more clearly understood by those within the profession as well as by those outside. This trend greatly accelerated after World War II because of the forester's more critical look at his resource. Revealed was an increasing demand for dwindling old-growth stumpage and for fast-maturing second-growth timber. The forester was looking ahead, too, at the staggering amount of wood that predicted future human populations would need. The most recent appraisal of American timber resources indicated that the country's future need for wood might be met only if the alarming rate of timber mortality and increment loss from forest insects could be checked. At present about 25 billion board feet of timber is either killed outright each year or prevented from growing because of forest insect infestations in all parts of the United States.[2]

Concern over the role of insects in the forest has become so great that foresters are demanding more efficient ways of detecting and controlling epidemic infestations. Forest entomologists can hope to meet these demands only by additional research, some of which has already

[1] Presented at the meeting of the Pacific Branch, Entomological Society of America, Portland, Oregon, June 27, 1957.

[2] U. S. Department of Agriculture, Forest Service, 1955. Timber Resource Review. Chapter IV, Factors Affecting Future Supply and Quality of Domestic Timber.

been catalogued. Experience gained from some of the recent large-scale forest insect control projects throughout the country has indicated a need for additional comprehensive research in several subjects: (1) insect biology and epidemiology, (2) forest insect survey methods, and (3) biological and applied control methods more specific to the pest insect and less harmful to the flora and other fauna of the forest environment.

Some of this research may require basic forestry knowledge such as that dealing with the timber damage aspects of insect appraisal surveys and some phases of insect control technique development. Some may require basic training in entomology. And some is almost certain to require the skills of such allied sciences as chemistry, plant physiology, pathology, and genetics.

As forest insect research delves into more basic problems, it is becoming apparent that some of it can be accomplished only by scientific teamwork. The complexity of the problems yet to be solved will require greater specialization of skills and more intensive training in these skills. It is doubtful if many of these problems can be resolved by technicians in any one of the scientific disciplines, as some of them formerly were by the forest entomologist—part forester, part entomologist. If diversification of forest insect research effort is to materialize further, the definition of a forest entomologist and the qualifications pertaining thereto will have to be recast.

In the future the title of "forest entomologist" may aptly apply to entomology graduates, with or without secondary training in limited forestry coursework, whose careers will be primarily in the zoological aspects of forest insect research. To acquaint him with his working environment, some knowledge of general forestry, forest protection, silviculture, and forest management would be desirable but not essential.

Under this definition, the forest entomologist may play a major role in planning and conducting forest insect investigations that deal with insect biology. He may need the help of the research forester in relating the effects of certain biological phenomena to the management of forest properties. He may make wider use of the services of insect toxicologists, pathologists, or of meteorologists in unearthing some of the factors involved in the natural or applied control of forest insects. In some cases, whole problems may be turned over to the technicians of allied sciences for investigation.

There are numerous existing examples of this teamwork in forest insect research. Mechanical engineers have helped forest entomologists develop equipment to regulate the droplet size, dispersal rate, and distribution pattern of aerially disseminated insecticides. Chemists have helped to formulate insecticides and to assay the quantity ingested or deposited in insect tissues following chemical control treatment. Fish biologists are giving increasing assistance in determination of dosage rates and modes of dispersal of forest insect aerial sprays so that damage to game fish populations resulting from spray deposits in forest streams can be kept to tolerable levels.

Since the forest entomologist must depend more upon the highly specialized skills of these other sciences, he will find that he must also be more highly skilled in the entomological disciplines. Gaining this will require a more specialized education and the opportunity in the field to devote more effective time to bona fide entomological problems. In so doing, he may lack the skills of some earlier forest entomologists to singly undertake some of the forestry aspects of related insect biological problems, but this lack will be more than compensated by the contributions of the research forester.

Re-emphasizing the redefining the educational qualifications and career functions of the professional forest entomologist will serve to clarify his status and thus to answer the title question. If these changes are made in the right direction, the forest entomologist will no longer be part forester, part entomologist. Instead, he will acquire stature and recognition as a full-fledged economic entomologist. In this new role he will be better able to serve the entomological needs of the forestry profession. Of equal importance, he will benefit from the stimulation and inspiration that will come from closer contact and better communication with technicians in the more closely related branches of entomology.

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NOTICE TO AUTHORS

On the inside of the front cover of the September, 1957, issue of the *Bulletin of the Entomological Society of America* (Vol. 3, No. 3) the policy of omitting the footnote designating papers for which part of the publication costs are paid (unless authors of such papers requested that it be included) was announced.

At the Annual Meeting held at Memphis, Tenn., Dec. 2–5, 1957, the Governing Board of the Society adopted a resolution requiring that papers published in the *Annals of the Entomological Society of America* and in the JOURNAL OF ECONOMIC ENTOMOLOGY heretofore carrying the footnote "Paid paper" be identified in the future by the following footnote: "Partial cost of publication of this paper was met by..... ....." (Appropriate information on agency, institution, etc., to be inserted.)

OBITUARIES

John V. Schaffner, Jr.
1888-1957

John V. Schaffner, Jr., formerly a member of the staff of the New Haven, Connecticut Laboratory, Division of Forest Insect Investigations, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, died at his home in Rochester, Vermont on November 26, 1957. Mr. Schaffner retired from the Federal Service in June, 1953. He had a long and fruitful career —over 40 years—as forest entomologist in the northeastern states. Born in Boston, Massachusetts, on June 26, 1888, he was first employed by the State of Massachusetts as deputy tree warden and gypsy moth inspector. He attended Massachusetts State College and in 1911 was appointed entomologist at the Federal Gypsy Moth Laboratory in Melrose Highlands, Massachusetts. In 1935 the laboratory was moved to New Haven, Connecticut, and Mr. Schaffner was a member of the staff there until the time of his retirement.

He was the author of numerous publications on the life history and habits of forest insects. Two of his most notable writings were U.S. Dept. Agric. Miscellaneous Publication No. 188 in 1934 on the "Macrolepidoptera and Their Parasites" and a companion article on the "Microlepidoptera and Their Parasites" which was submitted for publication in December, 1957.

Undoubtedly his greatest contribution to forest entomology was the part he played in compiling information for the book U. S. Dept. Agric. Miscellaneous Publication No. 657 "Insect Enemies of Eastern Forests." He wrote sections on Lepidoptera and Hymenoptera, he critically reviewed the entire book, and he prepared an "Index to Insects by Host Plants" which was published separately as a Supplement to the book. Here he had an opportunity to call on his vast knowledge, gained from personal experience in collecting and rearing many of the species he discussed. He was an entomologist of the old school, interested not only in the relatively few economic forest insect pests, but also the large numbers of uncommon species as well. Certainly no one else knew so much about so many insects that attack the forest and shade trees of northeastern United States. And he was equally familiar with the host plants of the insects involved. He was a great inspiration to his colleagues and will be keenly missed.

He was a member of the American Association of Economic Entomologists and the Society of American Foresters, a Mason and an active churchman.

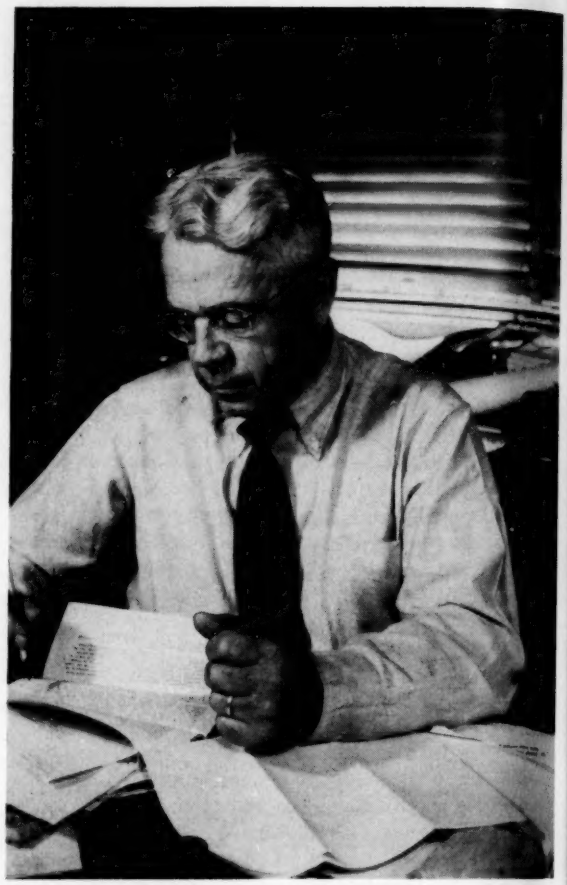
Beside his wife, Mrs. Annie Schaffner, of Rochester, Vermont, he is survived by three sons—Paul, a forester in the Philippines; Robert, an engineer in Boston, Mass.; John, a literary agent in New York City, and two daughters, Mary, a teacher in Philadelphia, Pa., and Eleanor who resides in Maryland.

RAY C. BROWN

Harry Scott Smith
1883-1957

Harry Scott Smith, Professor of Biological Control, Emeritus, at the University of California, passed away suddenly at his home in Riverside, California, on November 28, 1957.

Professor Smith was born at Aurora, Nebraska, November 29, 1883. His early life was spent on the farm and his education was obtained at the University of Nebraska, from which he received the A.B. and M.S. degrees in 1907 and 1908, respectively. Here he came under the influence of Lawrence Bruner and became his assistant for a short period after graduation. Then followed an appointment as Expert in the Bureau of Entomology, U. S. Department of Agriculture, first to study natural enemies of the cotton boll weevil, then the gypsy moth and finally the alfalfa weevil. On the last problem he travelled to Italy in search of natural enemies for importation into the United States. There he met the eminent Italian entomologist, Filippo Silvestri, and began a close association that continued until Silvestri's death in 1949.



Harry Scott Smith

In 1913 Smith was appointed Superintendent of the California State Insectary, then under the State Commission of Horticulture, with the primary responsibility of reorganizing the biological activities in the State and of placing that work on a sound scientific basis. This was accomplished to a substantial degree during the following 10 years, and in 1923 the research activities in biological control were transferred to the University of California. Professor Smith continued in charge of the Department until his retirement in 1951.

During his long period of service in directing the biological control work in California, Professor Smith developed the program for the mass production and utilization of *Cryptolaemus montrouzieri* in the control of the destructive mealybugs infesting the citrus orchards of the State. Mass production programs, perhaps best exemplified by that with *Macrocentrus ancylivorus* against the oriental fruit moth in 1944–46, were typical of his approach to many field problems in biological control.

The successes in biological control in California during this

period include complete suppression of the citrophilus mealybug through parasites imported from Australia in 1928, an achievement comparable to that of the control of the cottony cushion scale by the vedalia beetle 40 years before. The destructiveness of the black scale on citrus has been very greatly reduced through establishment of imported natural enemies, and the same is true of many less important pests.

To extend more fully the utilization of natural control agencies in pest control, the Laboratory of Insect Pathology, under Dr. E. A. Steinhaus, was set up within the Department in 1945, the first such unit in the United States. It has since attained world-wide recognition as a teaching and research center.

Professor Smith was responsible for the first attempt at biological control of weeds in the continental United States this being against the Klamath weed, *Hypericum perforatum*, in northern California. Leaf-feeding beetles of the genus *Chrysolina*, obtained from Australia, brought about a spectacular decline of this pest throughout the infested area.

Professor Smith's interests extended much beyond the field of biological control. The California plant quarantine system was under criticism at one time and its regulations were believed to be discriminatory against products from other states. A committee under the chairmanship of Professor Smith was appointed to study both the biological and economic aspects of the subject. The committee's report entitled "The Efficacy and Economic Effects of Plant Quarantines in California," published in 1933, brought the whole subject into proper focus and did much to standardize interstate plant quarantine practices.

Other interests included that of the development of resistance in insects to insecticides, which was the subject of his presidential address before the American Association of Economic Entomologists in 1940. Also, he was a keen student of ecology and population dynamics as bearing on biological control problems, and several of his papers on these subjects received wide recognition.

Professor Smith served as a member of the Invertebrate Consultant's Committee for the Pacific, Pacific Science Board, from 1948 and was a delegate to the International Congress of Entomology (1928) and the Pacific Science Congress (1933, 1949). He was a member of Sigma Xi, Delta Tau Delta, and Fellow of the American Association for the Advancement of Science. In the American Association of Economic Entomologists he served as vice-president in 1924, president in 1940, chairman of the Pacific Branch in 1924, and was elected to honorary membership in the Entomological Society of America in 1957. In recognition of his professional attainments the University of Nebraska conferred upon him the honorary degree of Doctor of Science in 1953.

Professor Smith is survived by his wife, Psyche Bruner Smith, five children and 10 grandchildren.

Prof. Harry, as he was affectionately known to his associates and a host of friends, will be long remembered for his never failing kindness and consideration for others and for his stimulating influence on all who were associated with him.

C. P. CLAUSEN
S. E. FLANDERS

BOOK REVIEWS

ATOMIC ENERGY IN AGRICULTURE by William E. Dick; 150 pages; $6.00; Philosophical Library, 1957.

William E. Dick obtained his subject matter from the international conference on the peaceful uses of atomic energy, which was held at Geneva in 1955. His book is written for general scientific readers, and it is pleasingly free of technical jargon. The recent uses of radioactive materials in plant breeding, tracer and tagging applications, and food sterilization are presented with dashes of colorful history. The importance of entomological research is recognized. Dick's expensive short book will inevitably be compared with C. L. Comar's "Atomic Energy and Agriculture" (reviewed in this *Journal* **51**(1): 116) which is a more technical presentation of essentially the same subjects. "Atomic Energy in Agriculture" is recommended for enjoyable informative reading; "Atomic Energy and Agriculture" is recommended for critical study or as a reference.

ROY J. BARKER

METHODS OF INSECT CONTROL, PARTS I AND II—REVISED. By DWIGHT ISELY. Braun-Brumfield, Inc., Ann Arbor, Michigan. vii+200 pages, 1957. Price, $3.50.

This book is unique among textbooks of applied entomology. The material presented is applicable to problems of insect control in any part of the world. The most important methods of control are presented in logical order with an analysis of the basic reasons why these methods are effective. Each control procedure is illustrated by the case method. Instead of attempting to list all the major pests with recommendations for their control given cookbook fashion, usually one species is thoroughly discussed to illustrate the method. It is the outgrowth of Professor Isely's more than 30 years of experience in all phases of applied entomology. This has allowed for exceptional discrimination in selection of the case problems used to illustrate the various methods of control. Presentation of the subject matter is characterized by conciseness and lucidity rarely found in works on applied entomology.

Emphasis upon analysis of insect problems makes the book especially effective. Principles are developed, based on the analyses, which do not change regardless of changes in practice resulting from new information. Soundness of these principles is demonstrated by the fact that there was no necessity for change in this edition of treatment given the basic principles of insect control in the first editions of Parts I and II. Development of almost incredibly effective insecticides during this period has brought about drastic changes in insect control procedures. Nevertheless, effective use of these new tools is still based on the fundamental principles discussed in this book.

The 43 chapters of Parts I and II of previous editions have been combined into one volume of 29 chapters. This has been accomplished by extensive reorganization of the text and reduction in subject matter. Having both parts combined into one volume is an improvement over the previous arrangement.

Each chapter is summarized concisely and contains a list of pertinent literature. The literature cited, though not exhaustive, includes most of the significant contributions to the subjects discussed.

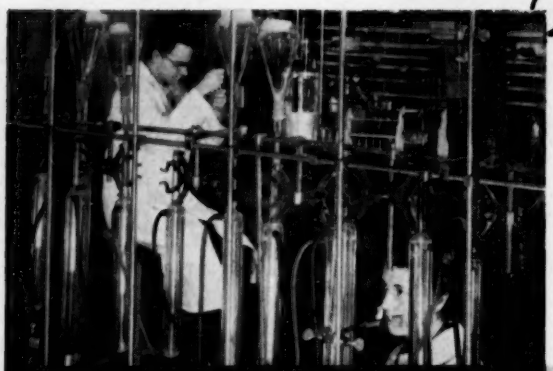
An unusually large number of typographical errors detracts from the overall excellence of the book.

Applied entomologists will find this to be an invaluable addition to their libraries, although the book is designed primarily as a textbook for a second course in entomology. It has much information which will be of interest to the less specialized members of the profession.

Familiarity with the basic principles of insect control presented in this book would do much to discourage the trend toward unsound recommendations which are based on "blind" or "automatic" schedules of insecticide application.

L. D. NEWSOM

What does it really take to kill insects?



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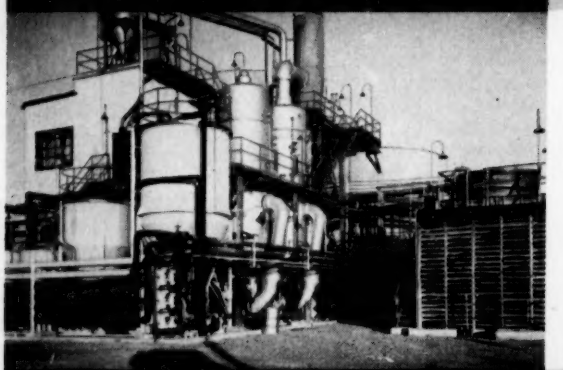
TESTING . . . years of field work, to determine effectiveness, dosages, side effects



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